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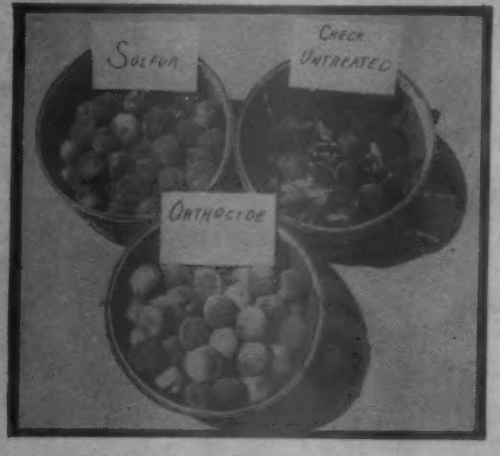

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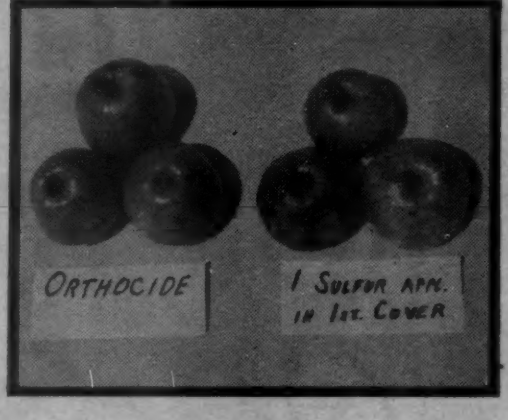

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GILBERT FRAZER MILES

W. H. Tisdale

Gilbert Frazer Miles, the son of Gilbert E. and Jennie Frazer Miles, was born June 23, 1893, in New York City and was graduated from high school in Hackensack, New Jersey. Although reared in a city environment, he displayed an early interest in agriculture. Specializing in horticulture at the Virginia Polytechnic Institute, he was graduated with the degree of Bachelor of Science in Agriculture in 1917, with honors, and as Valedictorian of his class. After a short period, during which he taught chemistry and engaged in research in plant pathology at his alma mater, Bert moved to Vanderbilt University where he organized the Military department. In 1918 he entered Officer Training School at Camp Gordon, Georgia. He finished the course with high honors and was retained for a time as military instructor.

Leaving military service, Miles joined the staff of the Agricultural Extension Service of the Pennsylvania State College. There he pioneered in the development of disease-free seed potato certification.

In 1926 Miles was employed by the Bayer Company as extension pathologist. In 1928, when the seed disinfectants interests of the Bayer Company were merged with those of E. I. DuPont de Nemours and Company, Miles became a member of the staff of the resulting company, Bayer-Semesan, and was later made pathologist in charge of the DuPont Minquadale Laboratory.

At the Minquadale Laboratory, Miles and his assistants collaborated with the chemists to make many fundamental contributions to the development of safer and more effective seed disinfectants and methods for their application. New Improved "Ceresan" and New Improved "Semesan, Jr." were among his early contributions. An effective dusting machine was developed to apply these and similar dusts. To meet the need for a highly effective treatment, free from irritation and vesicant action, Miles tested many new compounds synthesized for him by the DuPont chemists. The result was "Ceresan" M, the preferred formulation of N-(ethylmercuri)-*p*-toluene-sulfonanalide.

In the early nineteen thirties, the fungicidal properties of dithiocarbamic acid derivatives were discovered. After testing many of these derivatives for use as small grain seed disinfectants without success, Miles finally developed "Arasan," a formulation of bis(dimethylthiocarbaml) disulfide (tetramethylthiuram disulfide). A dispersible formulation, "Tersan", was subsequently prepared for spraying turf grasses.

To avoid the irritating features of "Arasan" when applied as a dust to seed corn, Miles helped to develop the slurry machine. The subsequent development of "Arasan" SF and SF-X by the chemists sparked the use of the slurry method more generally. Miles and his coworkers then developed "Arasan" M-2X for slurry application to other seed.

GILBERT FRAZER MILES
1893—1954

The trend toward the use of concentrated liquid organic mercurials offered a new challenge to Miles. Considerable success was being attained in this direction when Bert suffered a fatal heart attack while at work in his laboratory.

Miles also contributed substantially in developing better methods of experimenting with new seed disinfectants and soil fungicides and published a number of papers that deal with these subjects. He was made a member of the Advisory Board of the DuPont Agricultural News Letter in 1940. Here he served effectively in helping others with the publications.

Miles pursued his profession with earnest and intense devotion and was always ready to contribute where he could be of service. He worked long hours, probably ever mindful of the facts that led him to state—"Successful seed treating chemicals, like most other fungicides and insecticides, are rarely the result of a brilliant idea flashed from the brain of a genius. Rather, they are the result of the laborious, tedious, and monotonous examining, testing, comparing and studying of thousands of new chemicals."

PHYTOPATHOLOGY for December (44: 667-724) was issued December 30, 1954

SYMPTOMS INDUCED IN CHRYSANTHEMUMS ON INOCULATION WITH THE VIRUSES OF MOSAICS, ASPERMY, AND FLOWER DISTORTION[1]

Philip Brierley[2]

SUMMARY

In one experiment, 3 varieties of standard chrysanthemum, Friendly Rival, Good News, and Monument, were graft-inoculated with the viruses of mosaics and aspermy, singly and combined, and then flowered twice. In a second experiment, 1 variety, Friendly Rival, was graft-inoculated with the same viruses or combinations and also with a newly recognized flower-distortion virus, probably the one causing conspicuous symptoms in European chrysanthemum.

In ray florets, the mosaic viruses produced characteristic brown streaking which varied in intensity with the chrysanthemum variety and with the virulence of the mosaic virus introduced. Aspermy virus, which proved difficult to establish systemically in chrysanthemum, rarely produced leaf symptoms, but sometimes caused slight dwarfing of blooms together with waving and curling of the ray florets. The flower-distortion virus, found alone in the variety White Wonder and with aspermy and mosaic viruses in Yellow Spoon, caused severe dwarfing of flowers of Friendly Rival in 4 months and in Good News and Monument in 8–9 months. Ray florets were short, narrow, incurved, or irregularly curled.

European workers have attributed mosaic symptoms (1,8) and flower distortion (1,6,8) in chrysanthemum to the tomato aspermy virus. Prentice (7) pointed out that the aspermy virus is often associated with flower distortion but that it has not been shown to cause this symptom. He therefore proposed the name chrysanthemum flower distortion for the disease involving conspicuous symptoms on the flowers. In previous tests at Beltsville, a number of chrysanthemum varieties infected with aspermy and mosaic viruses in combination flowered without distortion of the blooms. Thus it seemed of interest to determine whether flower distortion is a reaction of certain chrysanthemum varieties to aspermy virus or whether an additional virus is concerned. Of 12 varieties listed by Bewley and Richards (1) as subject to flower distortion, only 2—Friendly Rival and Monument—are in general use in the United States. These varieties, as well as the variety Good News, were graft inoculated with mosaic viruses alone, with aspermy virus alone, and with mosaic and aspermy viruses in combination; they were then flowered twice after inoculation. In a second experiment, these viruses and virus combinations, as well as a flower-distortion virus previously unrecognized in the United States, were graft-inoculated into Friendly Rival; this series was flowered only once.

MATERIALS AND METHODS.—Virus-free test plants of the varieties Good News, Friendly Rival, and Monument were supplied by Yoder Brothers of Barberton, Ohio. All test chrysanthemum plants were grown in 6-in. pots in a greenhouse. Five plants of each variety were graft-inoculated from each virus source. The upper buds or lateral shoots were removed from the stock plants to promote growth of the scions, and lower lateral shoots were allowed to develop strongly before the plants were permitted to flower. Since all 3 test varieties are of the standard type, they were disbudded to permit development of 1 flower per shoot. Natural flowering was induced in November and December by shifting the plants to an unlighted greenhouse. After flowering, the plants were returned to a lighted greenhouse, pruned to 2 or 3 shoots and flowered again in the same pots in April and May under shade-cloth.

SOURCE OF VIRUSES.—Mosaic viruses B(6) or Q(5) were present in the following naturally infected chrysanthemum varieties, as determined by previous indexing on Good News chrysanthemum and on petunia. Crystal Valencia (B virus), Good News (Q virus), and Blanche (type source of Q virus) were from greenhouses in Ohio; Paris daisy (*Chrysanthemum frutescens* L.) (B virus) was obtained from a Maryland greenhouse, and Roza (B virus, from a Wyoming test nursery.

Mosaic and aspermy viruses combined were present in the following naturally infected chrysanthemum varieties and were determined by previous indexing on Good News, petunia, and tobacco. The varieties Nightingale and Lilac Mona Davis were from greenhouses in Ohio; Orchid Jewel and Yellow Spoon were garden varieties from a Pennsylvania nursery.

Tomato aspermy virus (2), not yet found occurring alone in chrysanthemum in United States (3,4), was introduced into the Good News scion variety by sap inoculation from tobacco. Only those Good News plants that yielded this virus on subinoculation to tobacco served as sources of scions. The 4 chrysanthemum varieties listed in parentheses in Tables 1 and 2 were the original sources of aspermy virus for these experiments. It proved difficult to produce systemic infection in chrysanthemum with the aspermy virus. Subinoculations often failed to detect this virus in stock varieties after months of contact with infected scions. Chrysanthemum plants inoculated with aspermy

[1] Accepted for publication August 18, 1954.

[2] Pathologist, Section of Ornamental Plants, Horticultural Crops Research Branch, Agricultural Research Service, United States Department of Agriculture, Beltsville, Maryland.

TABLE 1.—*Symptoms induced in Good News, Friendly Rival, and Monument chrysanthemums at second flowering April–May, 1954, following graft inoculation with viruses of mosaics, aspermy, or aspermy + mosaic, in July-September, 1953*

Virus introduced and variety source	Good News		Friendly Rival		Monument	
	Leaves	Flowers	Leaves	Flowers	Leaves	Flowers
Mosaic viruses:						
Crystal Valencia	Mild mottle	None	Veining	None	None	None
Paris daisy	Strong mottle	Severe streaking	None	None	None	None
Roza	Mild mottle	None	Veining	None	None	None
Good News	Mottle, crinkle; leaf dwarfing	Severe streaking	Veining	None	None	None
Blanche	Mottle, crinkle	Very late, small, severe burning (B+)[c]	Veining	Mild streaking	None	None
Mosaic + aspermy viruses:						
Nightingale	Mild mottle	Trace streaking	Veining (A+)[c]	None	None (A+)[c]	None
Lilac Mona Davis	Mottle, marginal crinkle	Severe streaking (A+)[c]	Veining (A+)	Smaller; trace streaking	None (A+)	Florets wavy, streaked (A+)[c]
Orchid Jewel	Mottle, marginal crinkle	Severe streaking	Veining (A−)	Smaller; trace streaking	None (A+)	None
Yellow Spoon[a]	Mottle, marginal crinkle	Very small, late, severe streaking	Veining (A+)	Very small, late, severe streaking (A+)[c]	Mottle (A+)	Small, late waviness, streaking (A+)
Aspermy virus[b]						
(Nightingale)	None (A+)[c]	None	None (A−)	None	None (A+)	None
(Lilac Mona Davis)	None (A−)	None	None (A+)	None (A+)	None (A−)	None
(Orchid Jewel)	None (A−)	None	None (A+)	None	None (A+)	Small, late (A−)
(Yellow Spoon)	None (A−)	None	None (A−)	None	None (A−)	Small, late

[a] Yellow Spoon infected with flower-distortion virus also.
[b] Scions of Good News chrysanthemums sap-inoculated from tobacco infected with aspermy virus from the source varieties listed in parentheses.
[c] (A+) tomato aspermy virus found, (A−) not found, (B+) B virus found, on subinoculation to petunia.

TABLE 2.—*Symptoms induced in Friendly Rival chrysanthemums graft-inoculated with viruses of mosaics, aspermy, and flower distortion January 21, 1954, and flowered May 17, 1954*

Virus introduced and variety source	Symptoms in leaves	Flower diameter (cm.)	Symptoms in flowers	Virus detected[d] on subinoculation
Mosaic viruses:				
Crystal Valencia	None	13	None	———
Paris daisy	None	13	None	———
Roza	Veining	13	None	———
Good News	Veining	13	Trace brown streaking	Flowers (B+)
Blanche	Veining	13	Trace brown streaking	———
Mosaic + aspermy viruses:				
Nightingale	Veining	13	Trace brown streaking	Flowers (—)
Lilac Mona Davis	Veining	13	Moderate brown streaking	Flowers (B+)
Orchid Jewel	Veining	13	Trace brown streaking	Leaves (B+)
Yellow Spoon[a]	Veining	8	Dwarfing; ray florets short, narrow, incurved	Flowers (B+)
Tomato aspermy virus:[b]				
(Nightingale)	None	13	None	Leaves (A+)
(Lilac Mona Davis)	None	13	None	Leaves (A+)
(Orchid Jewel)	None	13	None	Leaves (A+)
(Yellow Spoon)	None	13	None	Leaves (—)
Flower distortion virus:				
White Wonder	None	8	Dwarfing; ray florets short, narrow, incurved	Flowers (—)
Flower-distortion + mosaic viruses:				
Good News[c]	Veining	7	Dwarfing; ray florets short, narrow, incurved	Flowers (B+)
Flower-distortion + mosaic + aspermy viruses:				
Good News[c]	Veining	6	Dwarfing; ray florets short, narrow, incurved	Flowers (B+)

[a] Yellow Spoon infected with flower-distortion virus also.
[b] Scions of Good News chrysanthemum sap-inoculated from tobacco infected with aspermy virus from the source varieties listed in parentheses.
[c] Synthetic complexes in Good News Scions.
[d] (A+) tomato aspermy virus found, (B) B virus found, (—) neither found, on subinoculation to petunia.

virus but failing to yield this virus on subinoculation after the first flowering were then regrafted with the same source variety.

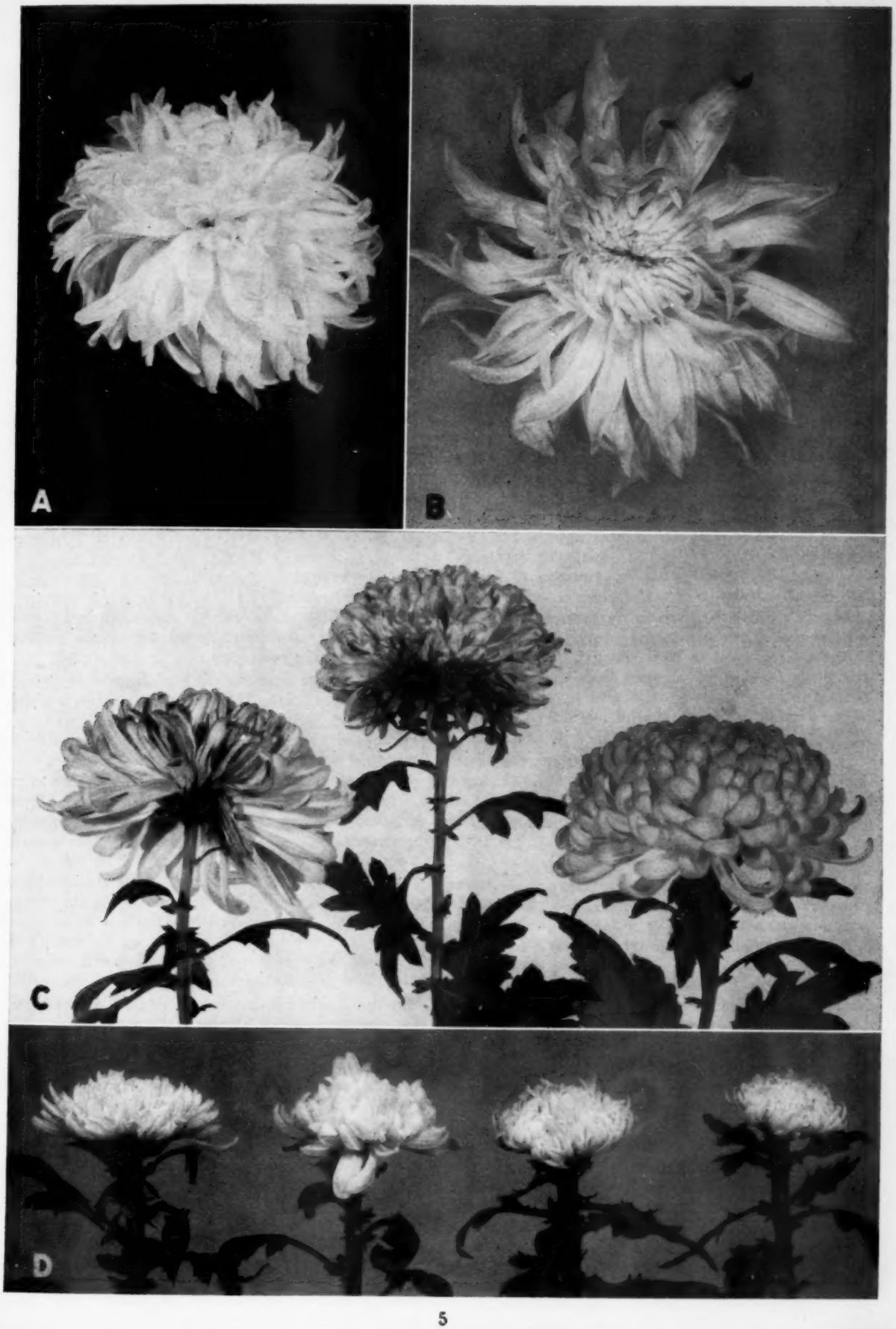
An additional virus included in the second experiment (Table 2) was chrysanthemum flower-distortion virus from the White Wonder variety from a Pennsylvania nursery. Synthetic complexes of flower-distortion virus with Good News Q virus and of aspermy with flower-distortion and Q viruses each in the Good News variety, were also tested on Friendly Rival chrysanthemums in this second experiment.

EXPERIMENTAL RESULTS. — Symptoms induced in Good News, Friendly Rival, and Monument following inoculation with viruses of mosaics and of aspermy and with combinations of these viruses in the first experiment are summarized in Table 1. Effects noted at first flowering in November and December, 1953, are not presented because these were in general similar but milder than the symptoms expressed at second flowering in April and May, 1954. Results of the second experiment, in which the viruses just listed and the flower-distortion virus were tested separately and together in Friendly Rival are summarized in Table 2.

SYMPTOMS OF MOSAICS.—The mosaic viruses, from naturally infected plants with long-standing infections, produced mild to severe leaf symptoms in Good News, a standard test plant for these viruses. The mosaic viruses from Crystal Valencia, Paris daisy and Roza are of Noordam's (6) B type, including local lesions only in petunia as he reported, and typical mosaic mottling without dwarfing or distortion of the leaf lamina in Good News. The viruses from Good News and Blanche are of Keller's (5) Q type, causing marked crinkling and leaf dwarfing in Good News and sometimes systemic yellow-blotched mottling in petunia. The Blanche mosaic virus was more damaging than Good News mosaic virus to the Good News

→

FIG. 1. Symptoms in chrysanthemum flowers caused by viruses. A) Tomato aspermy symptoms in Monument variety; B) in Good News variety; C) Brown streaking in Friendly Rival variety caused by mosaic, left and center, with normal flower, right; D) Symptoms of flower distortion in Friendly Rival variety; left to right, from Yellow Spoon (flower distortion, mosaic, and aspermy), from White Wonder (flower distortion alone), from White Wonder plus mosaic, and from White Wonder plus mosaic plus aspermy.


A
B
C
D

variety, causing severe brown necrosis of leaf and flower buds, dwarfing 3 of 5 plants to a height of 15 cm. at second flowering and killing 2 plants outright. The mosaic viruses produced no leaf symptoms in the Monument variety and only a mild chlorotic vein pattern in Friendly Rival.

The mosaic viruses produced a distinctive brown streaking in the ray florets of Good News and Friendly Rival (Fig. 1, C). This symptom was more consistently expressed in Good News than in Friendly Rival; in Monument it appeared only in plants inoculated with the viruses of mosaic and aspermy together. Streaking was more severe in Good News inoculated with the more severe mosaic viruses, which also induced stronger leaf symptoms. Good News mosaic in Good News delayed flowering 3 days, dwarfed the flowers to ⅔ normal size, and streaked the ray florets heavily throughout. Some of the milder B-type viruses, such as those from Paris daisy and Nightingale, also produced brown streaking, but the Crystal Valencia and Roza mosaic viruses did not. In the first experiment (Table 1) brown streaking appeared at both periods of flowering, November and December, 1953, and April and May, 1954; it also appeared in the second experiment in May, 1954 (Table 2). Streaking was also expressed in earlier experiments, always in the presence of mosaic infection. The B virus can be recovered by inoculation from such streaked flowers to petunia, unless the aspermy virus is also present; in that case as noted by Noordam (6) the earlier and stronger symptoms of aspermy hide the later and local expression of B virus. Brown streaking in ray florets is therefore a symptom of mosaic, but absence of streaking is not evidence of absence of mosaic.

Symptoms of aspermy.—In our experience, aspermy virus is typically symptomless in chrysanthemum leaves. Symptoms occasionally noted are white line patterns, diffuse chlorotic rings, and transitory chlorotic blotches suggestive of rings.

A distinctive flower symptom of aspermy is an irregular curling or waviness of the ray florets, with flowers 10-15 per cent smaller than normal ones. This flower symptom was not often expressed in these experiments because of the difficulty in establishing systemic infection with aspermy virus, but this virus was usually recovered from flowers that expressed it, and the symptom appeared only in plants inoculated with aspermy virus or with combinations that included aspermy virus. This symptom did not appear in the stock plants of Good News or Friendly Rival in either experiment; it appeared in 2 series of Monument plants inoculated with aspermy and mosaic viruses, (Table 1 and Fig. 1, A) and the aspermy virus was recovered from flowers of each of these series. The virus was introduced into these Monument plants in August and recovered by subinoculations in January. The plants were therefore not reinoculated in February, and aspermy infection was of 8 months' duration when these plants flowered in April. Although Friendly Rival graft-inoculated with aspermy virus in Good News scions developed normal blooms in December and May, the scion flowers were curled and waved at the May flowering (Fig. 1, B). At this time the scions had been infected for 9 months, but the Friendly Rival stocks for only 3 months, assuming that the first inoculation was unsuccessful, subinoculations being negative and the plants regrafted after first flowering. Our experience suggests that flower symptoms of aspermy are likely to appear only in plants of certain varieties that have been infected for long periods.

Symptoms of flower-distortion.—No distinctive leaf symptoms are known for the flower-distortion virus. The White Wonder variety passed all the usual index tests for other viruses of chrysanthemum, and was retained because of chlorotic speckling induced in leaves of Good News. When White Wonder infected with a flower distortion virus was grafted on Good News previously infected with B mosaic, the chlorotic spots tended to be somewhat larger and more distinct but were not constant in expression. Five plants of Good News grafted to White Wonder in January flowered without symptoms in April; they were not held for a second flowering. Thus it was not certain that White Wonder carried any virus until it was grafted on Friendly Rival (Table 2) and produced flower distortion. The Yellow Spoon variety also is symptomless for this virus; tests showed that it carries aspermy and mosaic viruses, but the presence of flower-distortion virus was unsuspected before the present experiments were completed.

In the first experiment (Table 1) Good News and Monument grafted to Yellow Spoon showed no flower distortion at first flowering, but 11 of 21 flowers of Friendly Rival had filiform and distorted ray florets. At second flowering, blooms of all 3 test varieties were small: 3–4 cm. in diameter in Good News, 4–5 cm. in Friendly Rival, and 7–8 cm. in Monument, 56–67 per cent smaller than normal ones. Ray florets were shorter and narrower than normal, incurved or irregularly curled. Brown necrosis of the ray florets, due to the mosaic virus also present, was prominent. Yellow Spoon thus induced a conspicuously more severe response in all 3 test varieties than did the other 3 sources of aspermy and mosaic viruses combined, indicating the presence of a third component virus in the Yellow Spoon source.

In the second experiment (Table 2) flower-distortion virus from White Wonder and combinations of this with mosaic and aspermy viruses, induced dwarfing (38–53 per cent) of Friendly Rival flowers comparable to that induced by the virus complex from Yellow Spoon (Fig. 1, D). In the same experiment (Table 2) mosaic and aspermy viruses, separately or together, induced no dwarfing of the flowers at first flowering. It seems clear that the third component in Yellow Spoon is the flower-distortion virus, which is present in White Wonder without other known viruses. Extreme dwarfing and distortion of the flowers of Friendly Rival at first flowering, 4 months after inoculation, are specific symptoms of the flower-distortion

virus. These symptoms are clearly expressed in the presence of mosaic or aspermy or both viruses. The brown streaking of mosaic accompanied flower-distortion symptoms when mosaic virus was present, but was lacking in the White Wonder culture, which is free from mosaic. Chrysanthemum stunt virus also graft-inoculated into Friendly Rival in this experiment, dwarfed the flowers only 19 per cent and caused no distortion or necrosis of ray florets.

DISCUSSION.—It is probable that the virus from White Wonder is the cause of the disease for which Prentice (7) proposed the name chrysanthemum flower-distortion. Although we have not duplicated the various symptoms of flower distortion illustrated by European workers, considerable variation in expression of this disease may be expected in different chrysanthemum varieties. Furthermore, European illustrations such as those of Bewley and Richards (1, Fig. 12), Noordam (6, Fig. 23), Prentice (7, Pl. II, Fig. 1), and Smith (9, Fig. 17) represent naturally infected plants which could have been infected with more than 1 virus.

For experimental analysis of virus complexes in chrysanthemum, grafting the unknown sample on the varieties Friendly Rival, Good News, and Mistletoe (Bronze, Golden, Pink, or White), and juice transmission to tobacco will detect the common viruses. Good News expresses leaf symptoms of mosaic viruses and Mistletoe leaf symptoms of stunt virus after 2 months or less. Friendly Rival must be flowered to detect the flower-distortion virus in the blooms after 4 months. These reactions are specific for the viruses named, and as far as known are not concealed or seriously disturbed by the presence of other viruses in the test sample. The chrysanthemum viruses juice-transmissible to tobacco are aspermy and tomato spotted wilt. They produce recognizably different symptoms.

Occurrence of the flower-distortion virus in American chrysthemums is disturbing. Specialists in the production of chrysanthemum cuttings for the florists' and nursery trade generally index their stocks by grafting to Blazing Gold or Mistletoe varieties to eliminate plants affected with stunt or mosaic. Some of them also use the juice-inoculation test on tobacco to eliminate carriers of aspermy. Our evidence shows that these tests are not effective in detecting the flower-distortion virus. The 2 varieties found infected with this virus are garden chrysanthemums. Nothing is known of the prevalence of flower-distortion in this country, but the lack of reports of conspicuous dwarfing of chrysanthemum flowers suggests that the disease is not common. The potential importance of flower-distortion in American chrysanthemums depends on whether the virus can be transmitted by a vector or by other natural means. The importance of aspermy and mosaic viruses is greater than we have recognized previously, inasmuch as these viruses can produce flower symptoms in varieties which express mild leaf symptoms or none.

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INHERITANCE OF RESISTANCE TO LOOSE SMUT OF WHEAT IN THE CROSSES OF KAWVALE X CLARKAN[1]

E. G. Heyne and E. D. Hansing[2]

SUMMARY

F_1, F_2, and F_3 data from reciprocal crosses of Kawvale (resistant) with Clarkan (susceptible) and F_3 data from the backcross progeny to each parent were studied for reaction to loose smut race 11. The resistance of Kawvale was dominant and probably due to at least 2 factors. A major factor gave high resistance, 0–10 per cent infection, and a minor factor or factors gave moderate resistance, 11–45 per cent infection. The combination of all factors gave immunity to race 11.

Inoculation of florets at the time of pollination indicated that the resistance to loose smut race 11 is controlled by the embryonic genetic constitution of the F_1 plant rather than the genetic constitution of the female parent.

No association was found between loose smut reaction and the reaction to leaf rust or awn expression.

Loose smut of wheat, caused by *Ustilago tritici* (Pers.) Rostr., is a common disease throughout the world. Tapke (13) listed 6 measures that had been tried for control of this disease: 1) selecting large kernels, 2) using seed more than 1 year old, 3) removing smutted plants from the field, 4) using clean seed, 5) treating the seed with hot water, hot air, and steam, alone or in some combination, and 6) selecting resistant strains and varieties. The last method is the most practical way to control loose smut of wheat. Therefore, when breeding for resistance to loose smut an understanding of the mode of inheritance of resistance to this disease is helpful. This investigation was conducted to determine the inheritance of resistance to loose smut contributed by Kawvale, a valuable parent in the Kansas wheat breeding program.

REVIEW OF LITERATURE.—A number of reports have been made concerning the reaction of wheat varieties. Bever (4) reported that Kawvale was highly resistant to all races of loose smut thus far described. Kawvale and its derivatives have been used extensively as parents in breeding wheat in Kansas. Pawnee, a selection from the cross, Kawvale × Tenmarq, was reported by Atkins (1) to be the only commercial hard red winter wheat variety resistant to loose smut in tests conducted from 1937 to 1942. Other non-commercial varieties were resistant in these tests. In a later report, Atkins et al (2) reported the reaction of 288 United States varieties and hybrid selections to loose smut.

Relatively few reports have been made concerning the inheritance of loose smut reaction in wheat. Piekenbrock (11) crossed the resistant variety Grüne Dame with the susceptible varieties Rümker's Sommer-Dickkopf and Rimpau's roter Schlanstedter. There were 16.4 per cent and 19.3 per cent, respectively, of resistant F_2 plants in these 2 crosses. Grevel's (6) studies supported Piekenbrock's conclusion that resistance to loose smut was inherited as a mendelian recessive. Rudorf and Rosenstiel (12) reported 29 resistant varieties among 86 wheat varieties tested in Argentina. In the F_3 generation of the cross 38 M. A. (resistant) × San Martin (susceptible) they observed that resistance probably was due to 3 recessive factors.

Larose and Vanderwalle (8) studied reciprocal crosses of 1 resistant variety with 3 susceptible varieties that were inoculated by brushing spores of loose smut on the stigmas while the glumes were open. All F_1 plants were resistant and most of the F_2 plants were smut free, indicating dominance for resistance. Mentana (susceptible) × Hybrid Federation-Khapli (resistant) was reported by Milan (9) as being highly resistant in the F_1 generation.

Caldwell and Compton (5) reported that the resistance of Trumbull was dominant and monogenic in the cross Wabash × Trumbull.

Tingey and Tolman (14) in an extensive study of the crosses Hope × Federation, Hope × Dicklow No. 3 and Preston × CI 11542 reported that Hope (immune) had 3 factors for resistance R_1R_1, R_2R_2, and R_3R_3; Preston (resistant) had the factors R_1R_1 and R_2R_2; CI 11542 had the factors R_2R_2 and R_3R_3 whereas Dicklow No. 3, the least resistant of the group, had only 1 factor, R_3R_3. Federation was susceptible. These 3 factors were incompletely dominant and had a cumulative effect.

MATERIALS AND METHODS.—Kawvale, CI 8180, an awned semi-hard red winter wheat selected from Indiana Swamp, is highly resistant to loose smut and resistant to race 9 of leaf rust. Clarkan, CI 8858, is an awnless soft red winter wheat that is highly susceptible to most races of smut and leaf rust occurring in Kansas.

The loose smut inoculum was Kansas collection 2 and identified as race 11 according to the classification of Bever (3). The inoculum was maintained on the variety Clarkan.

Reciprocal crosses were made between Kawvale and Clarkan in 1940. The F_1 plants were grown in the

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TABLE 1.—*The reaction to loose smut of Clarkan and Kawvale and their F_2 and F_3 progeny in 1942 and 1943*

Material Classified	Number of lines, rows, or plants in each smut percentage class 0	1–10	11–45	46–100	Total
Clarkan × Kawvale and reciprocal F_2 plants 1942	2068	0	0	196	2264
Clarkan × Kawvale and reciprocal F_3 lines 1943	581	326	249	66	1222
Clarkan, no. rows 1943	0	0	14	35	49
Kawvale, no. rows 1943	49	0	0	0	49

greenhouse in 1941 and 1 spike from each plant inoculated with loose smut by the partial vacuum method (10). The F_2 generation was grown along with the parents in 1942, plants being obtained from both inoculated and noninoculated seed. The F_2 plants from noninoculated seed and parental plants were inoculated with loose smut at anthesis by the partial vacuum method. The F_3 generation was grown in 1943 when a natural infection of leaf rust occurred.

Backcrosses to each parent were made in 1941 and the inoculated F_3 generation of the backcrosses studied in 1944. The original crosses were remade reciprocally in 1944 and dry chlamydospores of loose smut were placed in ½ the florets at the time of pollination and in the remaining half 24 hours later.

The F_3 lines were classified for percentage of plants infected with loose smut, awn expression (awned, awnless, or segregating), and leaf rust response (resistant, susceptible, or segregating). Each F_3 line or row of the parents contained about 30 plants.

EXPERIMENTAL RESULTS.—During the 4 years of this study, from 1942 to 1945 Kawvale showed no loose smut infection whereas 25–90 per cent of the plants in the Clarkan rows were infected. Nine Clarkan plants, 49 F_1 plants of Clarkan × Kawvale, and 50 F_1 plants of Kawvale × Clarkan were grown in 1945 from seed from florets that were hand pollinated and inoculated with dry chlamydospores. None of the F_1 plants showed loose smut infection whereas 7 of the 9 Clarkan plants or 77.8 per cent were infected. Kawvale was not inoculated. This response suggested that the resistance of Kawvale was transmitted as a dominant character and that the hybrid's resistance to infection was determined by the nature of the embryo, rather than by any response of the pistil and closely associated floral parts.

Data on reaction of the parents and hybrid progeny are given in Table 1. Only 196, or 8.7 per cent, of 2264 F_2 plants were infected with loose smut. No genetic explanation of the mode of inheritance could be made in this generation, but it confirms the evidence obtained in the F_1 generation that the resistance of Kawvale to race 11 is dominant to the susceptibility of Clarkan.

The average infection of 49 rows of Clarkan in 1943 was 59.2 ± 4.9 per cent. The frequency of F_3 lines in the infection class 46–50 per cent was less than in the lower infection classes and lines with 46 per cent or more of infected plants were considered equal to Clarkan in susceptibility. About $\frac{1}{16}$ (66) of the F_3 lines were within the infection range of the susceptible parent, whereas 581 were like Kawvale in having no loose smut infection. Bever (3) classified wheat varieties as resistant if they fell within the range of 0–10 per cent infection and 11 per cent or more as susceptible. If the 907 lines in the class 0–10 per cent infection are considered resistant and the other 315 lines in the 11–100 per cent infection classed as susceptible, the inheritance of loose smut reaction in this cross would be controlled by 1 major factor. However, Clarkan rows varied from 25–90 per cent of infected plants and Kawvale, like the F_1 hybrids, showed none. The presence of lines in the 11–45 per cent class may indicate more than 1 factor difference for loose smut reaction in this cross.

TABLE 2.—*The reaction to loose smut of Clarkan and Kawvale and the F_3 lines of the backcrosses to each parent in 1944*

Material Classified	Backcross plant no.	Number of lines or rows in each smut percentage class 0	1–10	11–45	46–100	Total
Clarkan 2 × Kawvale, F_3 lines	120	16	4	19	9	48
	123	12	3	18	12	45
	125	0	0	5	45	50
	126	0	0	5	38	43
	129	0	1	3	23	27
	131	18	6	9	18	51
Kawvale 2 × Clarkan, F_3 lines	96	17	11	6	2	36
	97	38	0	0	0	38
	102	52	0	0	0	52
	103	23	21	7	3	54
	110	41	0	0	0	41
	111	46	0	0	0	46
Clarkan, no. rows		0	0	2	34	36
Kawvale, no. rows		36	0	0	0	36

The smut reaction of F_3 lines of the backcrosses to each parent are given in Table 2. The progeny of only 6 F_1 backcross plants of each cross were studied in the F_3 generation. In the cross Clarkan 2 × Kawvale, the progeny of 3 plants, numbers 125, 126, and 129, could be considered within the range of the susceptible parent Clarkan. The progeny of the other 3 F_1 backcross plants numbers 120, 123, and 131 segregated for lines as resistant as Kawvale to those as susceptible as Clarkan. This is the result one would expect if 1 factor pair was involved for resistance to loose smut race 11 in this cross. In the 3 segregating families 46 lines had no smut, 13 had 1–10 per cent smutted plants, 46 had 11–45 per cent and 39 were similar to Clarkan with 46–100 per cent. One-fourth or 39 of 144 lines were susceptible. However, the ratio of 46:59:39 does not fit a 1-factor segregation nor does a 1-factor segregation satisfactorily explain the occurrence of lines showing

TABLE 3.—*Loose smut reaction of F_3 lines from the cross Kawvale × Clarkan and Kawvale 2 × Clarkan with observed and calculated numbers for a fit to a 12:3:1 ratio*

Smut infection classes				
0–10	11–45	46–100	Total	Range of P
Kawvale × Clarkan				
obs. 907	249	66	1222	
cal. 916.5	229.1	76.4		0.20–0.30
Kawvale 2 × Clarkan				
obs. 72	13	5	90	0.50–0.70
cal. 67.5	16.9	5.6		

resistance or moderate resistance (1–45 per cent infection).

The data from the backcross Kawvale 2 × Clarkan, on general appearance, also seem to fit a simple major factor explanation as 4 families, numbers 97, 102, 110, and 111, were resistant and the progeny of 2 families, numbers 96 and 103, segregated for loose smut reaction. The segregation of families 96 and 103 would be expected to be like the F_3 lines of the cross Kawvale × Clarkan. A similarity did occur as there were 40 lines with no smut, 32 with 1–10 per cent smutted plants, 13 with 11–45 per cent, and only 5 with more than 46 per cent; or approximately $\frac{1}{16}$ of 90 lines were susceptible like Clarkan.

Considering all the data obtained from the F_3 lines of Kawvale × Clarkan and reciprocal crosses and of the backcrosses to each parent, there appears to be at least 1 major factor and at least 1 minor factor for loose smut reaction contributed by Kawvale. The data are reassembled and presented in Table 3. The assumption has been made that 1 major factor and a minor factor in a heterozygous condition accounts for a level of resistance of 0–10 per cent infection. Although 99 F_1 plants, which were heterozygous, did not show any smut whereas Clarkan had 77.8 per cent smutted plants; there is still the possibility that some of these escaped infection because of their high level of resistance and/or the technique of inoculation used. Lines having all factors homozygous would be like Kawvale and show no infection. The separation into 2 classes did not seem feasible with the data available so the lines having 0–10 per cent of the plants infected were combined into 1 class. Lines showing 11–45 per cent infection were assumed to carry only the minor factor or factors while lines showing 46 or more per cent infection were assumed to have the susceptible genotype of Clarkan. The data obtained fit the hypothesis for a 12:3:1 ratio. Even though the data from the backcross Clarkan 2 × Kawvale do not exactly fit this hypothesis, the numbers of F_3 lines were low and possibly the F_1 backcross plants, numbers 120, 123, and 131, by chance, carried only the major factor from Kawvale. Thus a 2-factor hypothesis appears to be more plausible than a single factor explanation. Information from the practical breeding work in Kansas also gives some support to a 2-factor hypothesis.

Chi-square for independence for the association of loose smut reaction and awns was non-significant with a P value of 0.80–0.90 with 4 degrees of freedom. Likewise, there was no association of loose smut reaction and leaf rust reaction, as a P value for independence of 0.20–0.30 was obtained. Heyne and Livers (7) reported that the resistance to leaf rust race 9 in Pawnee, which was derived from Kawvale, is located on chromosome X. Also a major factor or awn expression of Pawnee (hdhd) is on chromosome VIII, so by analogy neither chromosome VIII nor X of Kawvale, a parent of Pawnee, carries the factors for loose smut resistance.

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A LEAF SPOT OF TOMATO CAUSED BY STEMPHYLIUM FLORIDANUM SP. NOV.[1]

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SUMMARY

Stemphylium floridanum sp. nov. is the casual agent of a tomato leaf spot very similar to gray leaf spot caused by *Stemphylium solani*. Morphologically, *S. floridanum* is characterized by oblong conidia that are several times longer than wide and roughly verrucose. Major cross septa vary from 1-6, though the average is 2-3. Conidiophores with swollen apical cells are extremely long and marked at intervals by 1-7 nodular swellings.

Growth in culture of the fungus occurred at 5°C. and 35°C., with an optimum of 26°C. Maximum spore production occurred at 23°C. A water soluble pigment varying from yellow to dark red is produced by the fungus when grown on potato dextrose agar and other media.

Experiments indicate that the fungus is pathogenic on a number of tomato varieties, pepper, wild soda apple and possibly gladiolus. Infections produced by the fungus develop most readily during the cooler months in Florida.

Conidia of the fungus have survived 4-month periods on dried plant debris and served as an efficient source of inoculum in greenhouse and field studies. Conidia have also survived 8 months in dried petri dish culture and produced vigorous infections.

Isolations made from lesions on tomato leaves, similar to those of gray leafspot, yielded a *Stemphylium* sp. different from *Stemphylium solani* Weber, the causal agent of gray leafspot. Numerous pure cultures were obtained from surface sterilized diseased tissue plantings on potato dextrose agar and by single spore technique. All resultant cultures were identical in appearance. A typical culture (E-8) originating from a single spore was selected to serve as the basis for all further studies.

Preliminary investigations indicated that the fungus is pathogenic to various commercial tomato varieties and differs significantly from described species of Stemphylium. A preliminary report of the disease was made in 1950 (3).

From observations and experiments conducted in the field, greenhouse, and laboratory and herewith reported, the organism is considered a new species and is designated as *Stemphylium floridanum* sp. nov.

HOST RANGE.—Natural infection by the fungus has been observed only on varieties of tomato (*Lycopersicon esculentum* L.). Experiments indicated that pepper (*Capsicum frutescens* L.) and wild soda apple (*Solanum aculeatissimum* Jacq.) are very susceptible. Inoculations of tobacco (*Nicotiana tabacum* L.), potato (*Solanum tuberosum* L.), petunia (*Petunia hybrida* Vilm.) and China-aster (*Callistephus chinensis* Nees) failed to result in infection.

DISTRIBUTION OF THE DISEASE.—Although the fungus originally was found in material collected at Gainesville, Florida, isolations of it were made also from diseased tomato plants collected at Bradenton, Florida, in July 1951. Recently a fungus isolated from diseased tomato leaves found in the Homestead, Florida, area proved to be the same organism.[3] Because of the similarity of symptoms produced by this fungus and those initiated by the gray leafspot organism, *Stemphylium solani* Weber, isolation in pure culture remains the only positive method of determining the presence of either *S. floridanum* or *S. solani* in tomato growing areas where *S. solani* is known to exist. So far as is known, the distribution of *S. floridanum* is limited to Florida, whereas *S. solani* has been reported from Florida (11, 12, 13), Georgia (2), South Carolina (2), Texas (15), Indiana (9), Ohio (9), and the Hawaiian Islands (4).

SYMPTOMOTOLOGY.—Plants in any phase of development may become infected by the fungus. Lesions are confined for the most part to the leaf blades but may occur on the petioles, stems, and sepals. No lesions have ever been found on fruits regardless of their stage of maturity. Lesions, when first observed on the leaflets, are small, glazed, irregular or circular areas 1–2 mm. in diameter and of a watery gray color. At this stage they are easily observed in transmitted light.

With age the lesions enlarge to 4 mm. or more in diameter, the centers become tan or brown while the margins often turn dark brown or black. Centers of lesions may crack and fall out to give leaflets a shothole appearance. Whole lesions may be surrounded by a narrow, chlorotic halo. Numerous small lesions may coalesce. All leaves may be infected; however, the largest lesions are formed on the older leaflets. In advanced stages of infection the leaves are chlorotic, flaccid and epinastic. Later they dry out and drop.

Lesions formed on stems and petioles usually are elongated or circular, slightly sunken areas varying from 2-10 mm. in length and 1-4 mm. in width. At first, the lesions often are difficult to distinguish from healthy tissue but with time the depressed areas dark-

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[3] Culture collected and supplied to the senior author by Dr. Grover Sowell, Gulf Coast Experiment Station, Bradenton, Florida.

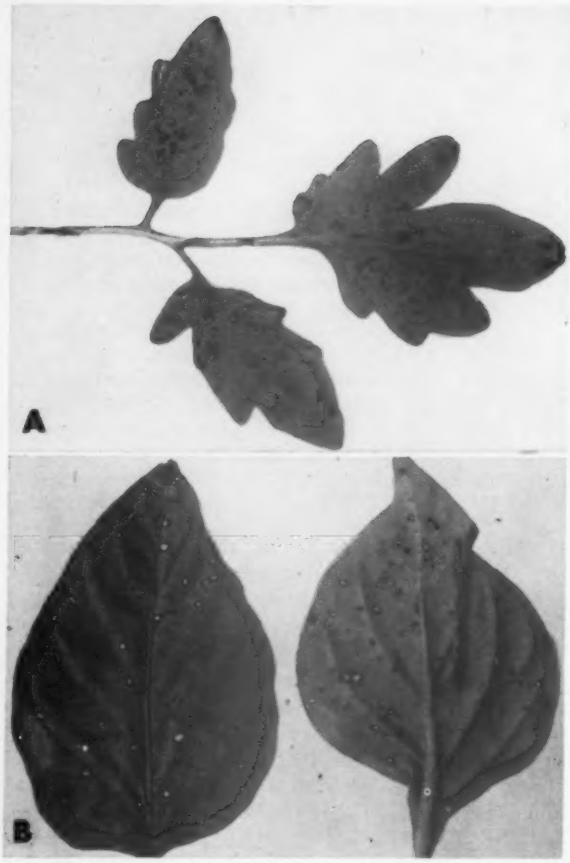

FIG. 1. Symptoms produced by inoculation with conidial suspensions of *Stemphylium floridanum* on A) tomato leaflet and B) pepper leaves.

en, become more noticeably sunken and often furrowed by cracks in the blackened, dead tissue (Fig. 1, A).

Signs.—Conidiophores and conidia are produced in quantity in brownish-black masses having a metallic lustre on the upper surface of lesions on fallen leaves lying on the soil. Conidial production is abundant also on lesions in leaves still on the plant when these leaves are subjected to high relative humidity. Conidia are also produced on lesions in the stems and petioles, providing that environmental conditions are favorable for spore production. Conidia can be observed in abundance on lesions in plants artificially inoculated and maintained in a moist chamber 2-5 days after the appearance of typical lesions. In the field the majority of spores are produced on the dead leaf debris lying on the soil or on epinastic lower leaves; in either instance the relative humidity, an important factor for spore production, is usually high.

THE PATHOGEN.—The fungus has been classified in the Eustemphylium group of the genus Stemphylium in accordance with the recent concept of the genus (14). The muriform conidia of this group vary in shape from ovate, subangular, sarciniform, or ellipsoidal with a prominently constricted median septum. In addition, the conidiophores have nodular swellings at irregular intervals along their length and a bulbous apical cell. Nodular swellings are darker in color because of a secondary wall formed in each swelling. Swellings themselves result from the conidiophore proliferating through its apex after a conidium has been shed (6, 13).

The conidia of *Stemphylium floridanum* are subangular, oblong, or rectangularly-oblong with conspicuous constrictions at the median septum and at one or more other septa. They are produced on long, rigid conidiophores, which have as many as 7 nodulose swellings spaced at irregular intervals along their length. No ascerigous stage of the fungus is known.

Two other Stemphylium species have been reported parasitic on tomato. Samuel (10), in South Australia, recorded a *Stemphylium* sp. causing a tomato fruit rot in nature. Its sarciniform conidia and *Pleospora* perfect stage separate it from *S. floridanum*. In addition, the latter fungus has never been associated with a fruit rot.

The second species parasitic on tomato is *S. solani* Weber, the causal organism of the gray leafspot disease. A single, deeply constricted median septum is

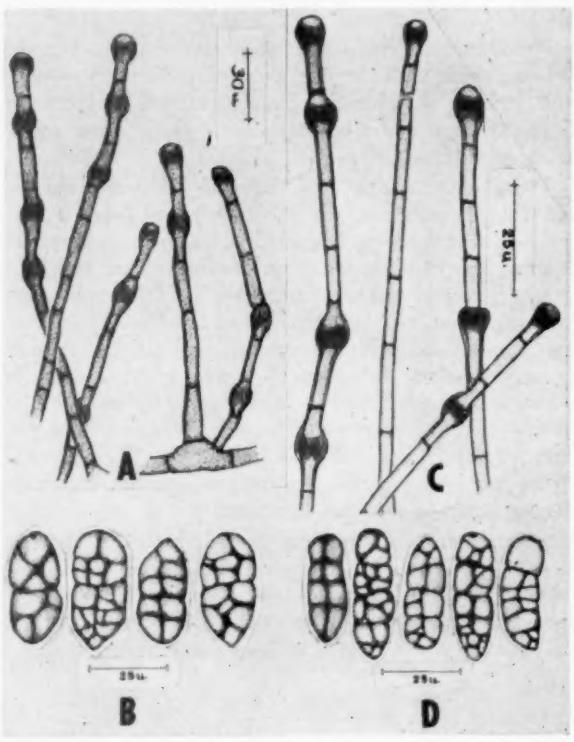

FIG. 2. Camera lucida drawings of conidia and conidiophores of *Stemphylium solani* and *Stemphylium floridanum* collected from naturally infected tomato plants: A) conidiophores of *S. solani*; B) conidia of *S. solani*; C) conidiophores of *S. floridanum*; D) conidia of *S. floridanum*.

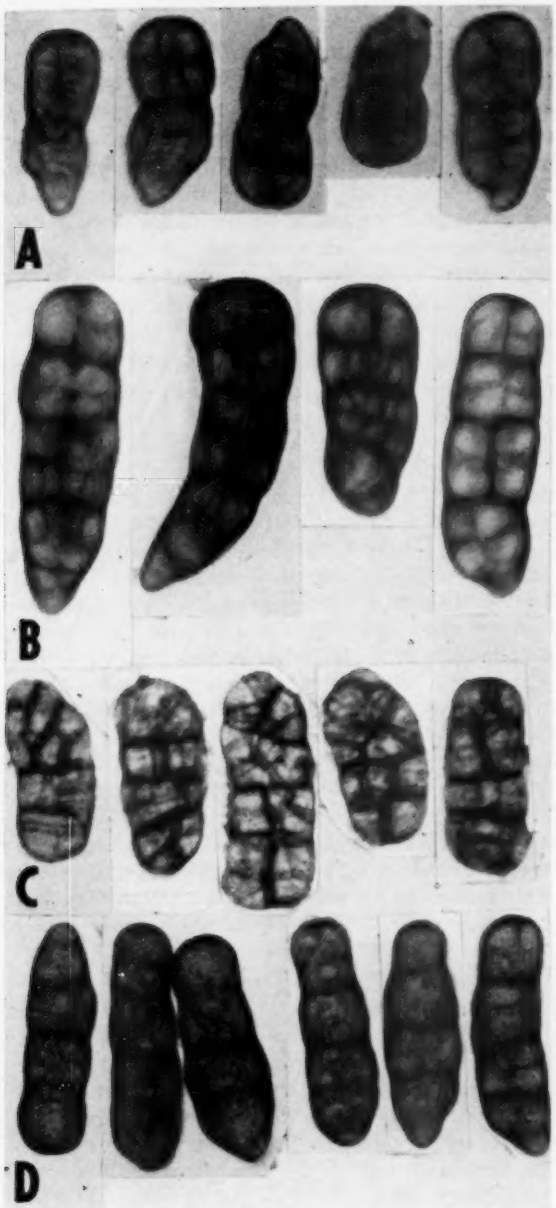

FIG. 3. Conidia of 4 different Stemphylium species taken from cultures grown 7 days on green-tomato dextrose agar (except B, which was grown on potato-2 per cent dextrose agar); A) *S. solani;* B) *S. callistephi;* C) *S. botryosum;* D) *S. floridanum.*

characteristic of the conidia of this species whether they are obtained from culture or field material (Fig. 2, B and 3, A).

Conversely, *S. floridanum* is not only constricted at the median septum but also at one or more other major cross septa (Fig. 2, D and 3, D).

Weber (11, 12) reported that 50 per cent of the conidia of *S. solani* measured 48 × 22μ. Measurements made by the senior author of 150 conidia of the fungus collected from naturally infected field tomato plants averaged 37 × 18μ. Hence the ratio of length to width is approximately 2:1. Conidia of *S. solani* collected from potato-dextrose and green tomato dextrose agar[4] cultures of the fungus give a similar ratio. Measurements of 250 conidia of *S. floridanum* from naturally infected material averaged 36 × 12μ for a length to width ratio of 3:1. The ratio increases to 5:1 with conidia collected from cultures of the organism grown on potato-dextrose agar.

Conidiophores of *S. floridanum* are 75–300μ long with a diameter of 3.0–5.5μ. Segments of some conidiophores have been measured that are over 350μ in length. Conidiophores are slightly darker in color than the vegetative mycelium and are often guttulate. Branches, when they occur in culture, originate at the base of the conidiophore or at one of the nodular swellings. Along the length of the conidiophore, spaced at intervals of 15–50μ, are 1–7 bulbous or nodulose swellings 6–8μ in diameter. A secondary wall forms in each of the swellings by the proliferation of the conidiophore after a conidium is shed. This accounts for their dark color (14).

The original illustrations by Weber (12) of *S. solani* give no indication of nodular swellings on the conidiophores. Of the herbarium and field material of *S. solani* examined, nodular swellings 6–8μ in diameter were apparent on all but the youngest immature conidiophores. Mature conidiophores of *S. solani* are shorter than those of *S. floridanum* and have fewer nodular swellings (Fig. 2,A and C).

Conidia of *S. floridanum* differ from those of *S. callistephi,* the causal agent of China-aster leaf spot described by Baker and Davis (1) in that the conidia of the latter produced on diseased leaves, have a length to width ratio of 2:1, which corresponds to that for *S. solani.* Further, the conidia and conidiophores of *S. callistephi* are very much darker in color than are those of *S. floridanum* and the conidia have smooth walls compared to the roughly verrucose wall of *S. floridanum.* In addition, a noticeable curvature seems to be characteristic of *S. callistephi* conidia. Attempts to infect China-aster with *S. floridanum* failed and, conversely, *S. callistephi* failed to infect tomato plants.

That the fungus isolated in Florida is not *S. botryosum* (Wall.) Ness is indicated by the difference in size and shape of conidia. Conidia of the type material measured by Wiltshire (14) were 24–39μ long × 19–31μ wide. Neergaard (6) has reported measurements of 13.5–66μ long × 7–28.5μ wide. According to Groves[5] "most spores of *S. botryosum* are 18–35 × 15–22μ." Conidiophores of *S. botryosum* are short and have few nodular swellings (6, 13). A culture of *S. botryosum* isolated from a tomato fruit rot lesion pro-

[4] Green tops of tomato plants, 250 gm.; dextrose 20 gm.; agar 20 gm.; water 1 liter.

[5] Letter from J. Walton Groves, Head Mycology Unit, Department of Agriculture, Ottawa, Ontario. November 27, 1952.

duced an ascigerous stage identified as *Pleospora herbarum* (Persoon) Rabenhorst on potato-dextrose agar cultures stored several weeks at 3°C. Attempts to infect tomato plants with conidia of this isolate were not successful.

Because of these differences and because of a wide latitude of differences between *S. floridanum* and other described species of Stemphylium, the fungus is herewith described as a new species.

Stemphylium floridanum sp. nov.—Hyphae hyalinae, olivaceae, ramosae, septatae, 5–9μ diam. Conidiophora subhyalina, olivacea, septata, ramosa, raro ad basem, rigida, non-fasciculata, 75–300μ × 3.0–5.5μ diam., apices bulbosi, 6–8μ diam.; tumores manifesti 1–7 ad intervalla 15–50μ. Tumores nodulose et apice fusci. Bases conidiophorum enormes. Conidia muriforma, acrogenes, solitaris, olivacea, fulva aut fusca, oblonga ad cylindrata, subangulara; ad apicum subacuta, acuta, circa, saepe rostrata; ad basium rotunda aut subtrauncata saepe cum ciratrica promines; septa transversa 1–6 (medio 2–3) profunde constricta; septa longitudinalia numero variabilia, nulla ad multa; muri saepe verrucosa. Conidia 19.9–62.2 × 7.6–23.0μ, fere 2–5 spatina longioria guan lata. Satus perfectus ignatus.

Hab. in folio vivo *Lycopersiconis esculentis*, Gainesville, Florida, U. S. A.

Hyphae hyaline, to slightly olivaceous, branched septate, 5–9μ in diameter. Conidiophores darker than vegetative hyphae, subhyaline, olivaceous, fuscous, septate, seldom branched, nonfasciculate, rigid, 75–300μ or more long, 3.0–5.5μ in diameter, with bulbous apexes 6–8μ in diameter; 1–7 conspicuous nodular swellings and apical swelling darker than remainder of conidiophore. Bases of conidiophores irregularly shaped. Conidia produced acrogenously; olivaceous, light brown or darker, muriform oblong, rectangular, or cylindrical, subangular; rounded or acutely pointed at apex, often rostrate; bases truncate or rounded often with prominent basal scars; conidia walls often roughly verrucose; transverse septa 1–6 (usually 2–3) deeply constricted; longitudinal septa none to many, constricted. Conidia 19.9–62.2 × 7.6–23μ usually 3–5 times longer than wide. Ascigerous stage unknown. Isolated from Stone Improved, Red Cherry, Yellow Plum and Rutgers varieties of tomato at Gainesville, Florida.

Type material has been deposited in the Herbarium of the Florida Agricultural Experiment Station, University of Florida, as item F-42672.

Potato-dextrose agar has been a very suitable medium for culturing *S. floridanum*. Spore production is abundant and the fungus will cover the area of a 90 mm. petri dish in 14 days at room temperatures. Czapek-Dox agar has also served as a desirable culture medium. Spore production and mycelial growth is restricted to varying degrees on Difco corn meal, purne, bean pod, and lima bean agar.

In cultures 10–12 days old or even in relatively fresh cultures, patches of white, grayish, or pink mycelium arise in the older areas back from the margins of the colony. Hyphae of this mycelium are branched, septate, hyaline, 2–4 mm. in diameter and produce an amber exudate which collects in droplets in the mycelium. Saltant mycelium grows rapidly, is dense, sterile, and may in time completely cover the original culture. When first observed, this condition was thought to be a contamination but cultures made from single spore isolations produced the same type of sterile growth. Except for the appearance of sterile saltants, cultures of *S. floridanum* have remained consistently similar on various media.

Beginning with the original isolate of *S. floridanum*, a characteristic pigmentation has been consistently associated with the fungus after 3–5 days' growth on potato-dextrose agar. Pigmentation begins at the center of the cultures as a faint martis yellow, as illustrated by Pl. 9, I-1, Maerz and Paul (5), which diffuses beyond the margins of the fungal growth. With continued growth of the fungus, the color darkens, with red becoming the predominant color. After 14 days' growth at room temperature, the color of the pigment resembles India Red-Arabian Red + Red Robbin—of Pl. 7, L-6, Maerz and Paul, (5). The amount and color of the pigment *S. floridanum* produces will vary with the medium on which it is grown. Pigmentation is copious on potato-dextrose agar but suppressed on Czapek-Dox agar. Cultures on potato-dextrose agar having less than 0.5 per cent dextrose failed to produce a pigment and the mycelium of such cultures was white. Investigations regarding the nature of the pigment have not been undertaken other than to establish its water solubility. Pigment production by *S. solani* and *S. callistephi* has not been reported.

Petri plates of potato-dextrose agar, pH 5.7, were infested with 5 mm. disks of the fungus, to determine the effect of temperature on the growth of the fungus. Five plates each of the fungus were incubated at 5, 10, 15, 20, 23, 26, 29, 32 and 35°C. for a period of 10 days before observations were made. Mycelial growth, though severely restricted, was not inhibited at temperatures of 5° or 35°C. Conidia were produced at temperatures from 5°–32°C. but optimum sporulation occurred at 23°C. and optimum mycelial growth between 26° and 29°C. Sterile, pink sectors appeared at 23°C. and were evident at all temperatures through 35°C.

Effect of hydrogen ion concentrations was determined by infesting petri plates of buffered potato-dextrose agar adjusted to 12 various pH's between 4.6–9.2. Five plate replicates were used for each specific pH. Incubation was at 23°C. After 10 days' growth, observations were completed. Growth of the fungus was noticeably restricted below pH 5.9 and was inhibited completely below pH 4.8. Media of near neutrality or slightly alkaline reaction seemed most conducive to growth whereas only a slight inhibition of the fungus was observed as alkalinity increased to pH 9.2. Optimum growth occurred at pH 8.0. In one

experiment restricted growth occurred at pH 10. Sporulation was abundant in cultures above pH 4.9.

Cultures kept in diffuse light in the laboratory produced abundant growth of the sterile pink mycelium but failed to produce conidia. An experiment designed to show the effect of light on sporulation indicated that cultures growing at room temperatures on potato-dextrose agar under normal lighting conditions produced conidia in conspicuous zones. Those wrapped in paper bags and incubated in a dark cupboard showed little evidence of spore production. Other cultures, incubated under constant illumination provided by normal daylight in the laboratory plus the light of a 60-watt incandescent bulb at night, showed abundant sporulation over the entire culture with no evidence of zonation. All petri plates retained their glass covers thus eliminating the stimulating effects of ultra violet light. These results coincide with those reported by Weber et al (12) working with *S. solani*.

PATHOGENICITY STUDIES.—*Stemphylium floridanum* was consistently associated with and isolated from lesions on leaves, stems, petioles, and flower calyxes. Conidia produced by several cultures originating from these sources, in addition to subcultures of the typical single spore isolate (E-8), were used in inoculation experiments in studying the pathogenicity and host range of the organism. All inoculum was grown on potato-dextrose agar at room temperatures for periods of 10–14 days. In preparing the spore suspension, sterile water was poured into the petri dish cultures and the conidia were loosened from the mycelium with a camel-hair brush. Mycelium was separated from the conidia by straining the suspensions through a fine wire mesh screen. To each 25 ml. of inoculum, a small platinum loop of detergent Tween-80 was added to reduce surface tension between the host plant and spore suspension. Plants inoculated with spore suspensions minus Tween-80 became diseased but because the suspension gathered into large droplets on the waxy leaf surface, the lesions produced were abnormally large and atypically shaped. The viability of all inocula used was tested by atomizing a small amount on petri dishes of water agar and observing germination. In no instance where Tween-80 was used did the percentage of spore germination rank below that of conidia in simple water suspension.

Inoculations were conducted on plants in various phases of growth, ranging from the cotyledon to flower and fruit stage. Inoculum was atomized thoroughly on all above-ground parts of healthy, non-wounded plants. Following application of inoculum, the plants were placed in moist chambers for periods of 12–48 hours, after which they were placed on the greenhouse bench for observation. Within the following 1–3 days, symptoms began to appear regardless of the stage of development of the plant when inoculated. Isolations made from lesions of inoculated plants yielded a fungus that produced spores identical with the inocula. Thus the pathogenicity of *S. floridanum* has been established.

TABLE 1. *Reaction of suspected host plants to inoculation with conidial suspensions of Stemphylium floridanum*

Host plant	Plants Inoculated	Percentage Infected
Lycopersicon esculentum L.	247	100
Solanum aculeatissimum Jacq.	21	100
Solanum gracile Link.	14	0
Solanum melongena L.	30	10
Solanum tuberosum L.	11	0
Capsicum frutescens L.	20	100
Petunia hybrida Vilm.	6	0
Gladiolus var. Pink Dubonair	62	16
var. Picardy	111	50

John Baer and Rutgers tomato varieties were used in most of the inoculation experiments. Varieties Stone Improved, Ponderosa, Southland, Lakeland, Pan American, New Stone, Grothem Red Globe, Marglobe, Red Cherry, and Yellow Plum were equally susceptible. The results of inoculating other suspected host plants are in Table 1.

Symptoms on *S. aculeatissimum* developed in much the same manner as those produced on tomato but the lesions were larger. No difficulty was experienced in recovering the organism from the lesions. Conidia produced on dead and dying leaves initiated secondary infection on terminal leaves.

On eggplant, the lesions remained small and were not surrounded by a chlorotic band. On pepper, symptoms first appeared as more or less circular, sunken, brown areas 0.5–2.0 mm. in diameter. In time, they enlarged to 3–4 mm.; the centers became dry and bleached to a very light tan or white, whereas the margins remained dark brown or black (Fig. 1, B). The fungus was easily reisolated from infected leaves.

Lesions appearing on inoculated gladiolus plants were small, sunken, circular, reddish-tan areas 0.5–2.0 mm. in diameter and resembled lesions described by Nelson (7). After 2 weeks, no further development in lesion size was observed. No attempts were made to reisolate the fungus from these lesions. Tomato plants inoculated with an unidentified *Stemphylium* sp.[6] associated with the red spot disease of gladiolus in Florida failed to cause infection.

Bearing tomato plants inoculated in the field under natural conditions with a spore suspension began to show symptoms within 4–7 days. Conidia produced on dead leaves lying on the soil served as a source of inoculum for secondary infections, in which case the symptoms that appeared on terminal leaves were similar to those of the primary infection. Although leaves, sepals and, occasionally, stems and petioles became infected, fruits remained healthy. Non-inoculated peppers growing adjacent to the infected tomato plants became diseased.

[6] Culture of *Stemphylium* sp. isolated from gladiolus supplied by Dr. R. O. Magie, Gulf Coast Experiment Station, Bradenton, Florida.

Stemphylium floridanum has never been observed to cause infection of fruit. To determine susceptibility of fruit, surface sterilized fruit were divided into 4 groups and inoculated by atomizing them with a spore suspension of the fungus. With a sterile scalpel, 3 wounds were made in each fruit of a lot of 20 green-wrap and 20 red-ripe fruit, while 20 green-wrap and 20 red-ripe fruit remained unwounded. The inoculated fruit were kept 1 week in the laboratory in glass moist chambers at room temperature. No infection occurred on the nonwounded fruit in either stage of maturity. A slight mycelial growth was noted at the peduncle scar on some fruit 3 days after inoculation but no lesions developed. All wound-treated fruit were infected only in the regions of the wounds. No lesions attributable to direct penetration of the fungus were evident. Infection of the wounded green fruit was restricted to the immediate area of the wound whereas infection of wounded ripe fruit involved areas up to 1 cm. around the original wound; there was an abundant production of surface mycelium and spores.

Plants may be infected by the conidia produced on tomato leaf debris as demonstrated by inoculating under both greenhouse and field conditions with crushed, dried, diseased leaves that had been stored in the laboratory 4 months at room temperatures. On greenhouse plants, typical lesions developed 72 hours after removing plants from the moist chamber. Under field conditions, lesions developed in 5-7 days. Leaves of plants growing in the field inoculated in the manner described were not moistened artificially. Dew supplied the moisture film in which the spores germinated. Conidia produced on infected leaves of *Solanum aculeatissimum* and pepper also served as similar inoculum in greenhouse experiments. Conidia of *Stemphylium floridanum* have also survived 8 months in dried petri dish cultures. Thus, it is reasonable to assume that conidia on plant debris in the field and those produced on wild host plants could serve as the chief source of inoculum for initial infections of plants in the field.

Seasonal relationship.—Inoculations with the fungus conducted under both greenhouse and field conditions caused infection of healthy plants most readily during the cooler months of the year, namely, from October to June. Little infection resulted when field and greenhouse inoculations were made the latter part of June, the months of July and August, and most of September. Climatic conditions at that time of year were extremely dry and warm and greenhouse temperatures ranged from 85°–116°F. Unsprayed tomato and pepper plants in the field plot showed no symptoms of other leaf diseases during the summer. These observations would seem to indicate that *S. floridanum*, like *S. solani* can be expected to cause most severe infections to tomato grown in Florida during the cool fall, winter, and spring months.

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EFFECT OF SEED MATURATION ON INHIBITION OF SOUTHERN BEAN MOSAIC VIRUS IN BEAN[1]

Pen Ching Cheo[2]

SUMMARY

Virus of southern bean mosaic was present in all flower and fruit parts of systemically infected bean varieties. Sepals, petals, and young fruits contained as much virus as infected leaves. Both seed coat and embryo contained virus. As seed matured, the virus content of the embryo increased while that of the seed coats and the pod decreased. Virus in the embryo was inhibited very rapidly when dehydration of the seed occurred, but not in seed coats and leaves. This inhibition may be due to certain chemical changes produced during the final stages of ripening.

Crude extracts from bean seed contained a virus inhibitor when added to southern bean mosaic virus. Extracts from mature or germinated seed caused greater inhibition than extracts from immature seed. Diluting the seed extracts reduced the inhibiting effect on the virus. The seed extract had no effect on susceptibility of the host cells to infection. The inhibiting substance in mature bean seed was heat stable and resistant to both acid and alkali.

Zaumeyer and Harter (13) reported that one of the differences between the viruses of southern bean mosaic and common bean mosaic is that the latter is seed-transmissible whereas the former is not. They showed that the virus of southern bean mosaic was present in fairly high concentrations in seed of systemically infected bean plants in early stages of development, but was apparently inactivated in later stages of ripening and storage. This paper deals with further investigations on the relation of maturity of bean seed to presence of southern bean mosaic virus.

MATERIALS AND METHODS.—Studies were conducted under greenhouse conditions at Beltsville, Md., at temperatures ranging from approximately 18° to 27°C. Kentucky Wonder bean (10) (Phaseolus vulgaris L.) was used as the local-lesion assay host for quantitative studies of virus concentration[3] in different plant parts. For virus assay comparisons, one of the simple leaves of a seedling was inoculated with expressed juice from one test sample and the other with juice from another sample. Neutral phosphate buffer solution was added to the inoculum and finely ground carborundum powder was used as an abrasive for the inoculations. The inoculum was applied to the leaves with a glass spatula and the leaves were rinsed with water immediately after inoculation. When comparisons of virus contents of different plant parts were made, the simple leaves of at least 10 seedling plants were inoculated.

RESULTS.—*Distribution of virus in flower and fruit parts.*—Assay of different parts of the flower and fruit of systemically infected bean plants showed that the virus was present in all flower and fruit parts. Juice from sepals and petals of infected plants had as high virus concentration as juice from young infected leaves. The average number of local lesions produced on Kentucky Wonder bean by extracts of sepals and petals was 230 per leaf, whereas 215 lesions per leaf were produced by similar extracts from young leaves. The juice from very young fruit (about 2 days after petal fall) contained about the same virus concentration as the flower parts, if not a higher one, and maintained it for a considerable period. Virus concentration decreased as the pod aged. After the pod had become yellow, leathery, and dry, the virus content was appreciably reduced. The same was true for seed, which even after reaching maturity contained a relatively higher virus concentration than the pod tissue. On a weight basis, young seed contained more virus than older ones, and the former had a greater virus content than the enveloping pod tissue. Seed coats contained more virus than the embryos and the concentration of virus in the coats of young seed was higher than in the coats of older seed. In one test, the concentration in the placenta was about the same as in the seed coats.

The concentration of virus in the embryo was lower than in other plant parts. There appear, however, to be varietal differences in this respect. Embryos from infected plants of Logan bean had a higher virus concentration than those from the Unrivalled Wax variety. Extracts from embryos of Logan produced 350 local lesions per leaf on inoculated Kentucky Wonder leaves as compared with 3 lesions per leaf from similar embryo extracts from Unrivalled Wax. There appears to be some evidence of virus multiplication within the embryo during maturation. After the seed were fully mature, colored, and dried, the virus was not found in either cotyledons or plumule.

Inhibition of the virus in infected seed.—Since the virus of southern bean mosaic was found in the embryos of immature seed, studies were made to determine whether a gradual or sudden reduction in virus concentration occurred as the seed matured. Seed from systemically infected plants were harvested at

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The author's services were available for this work through a grant of the Department of State.

[3] The term virus concentration in this paper refers to the active virus as measured by local lesion count.

TABLE 1.—*Interrelation of seed age, embryo weight, and infectivity of southern bean mosaic virus in Logan bean seed as measured by the number of local lesions produced on primary leaves of Kentucky Wonder bean*

Age of seed (days after blooming)	Weight of embryo sample	Local lesions per leaf produced by inoculum from: Embryo sample	Local lesions per leaf produced by inoculum from: Comparable leaf sample (standard)
	Gm.	No.	No.
19	0.18	16	502
24	0.54	29	469
27	0.79	88	256
30	1.08	130	448
32	0.90	286	375
35	1.33	284	270
38	1.50	360	457
40	1.53	480	328
43	1.55	582	399
47	0.98	2	355
49	0.78	0	233
51	0.78	0	365

different stages of maturity and the embryos were removed from the seed coats. Virus assays were made from these and compared with a standard inoculum prepared from young systemically infected leaves (1-200 dilution), stored in small glass vials, and kept frozen in a refrigerator. For each assay, 3 embryos of the same maturity were weighed and later ground in 6 ml. of neutral phosphate buffer. The relation between virus concentration and weight of embryo at different stages of maturity is shown in Table 1. Virus in the embryo disappeared very rapidly when dehydration of the seed occurred, and 43 days after blooming an average of 582 local lesions per leaf was produced by extracts of the embryo as compared with 399 lesions by leaf extracts. Dehydration of the seed had not taken place at this time. Four days later, when the embryos had decreased in weight because of dehydration, the number of local lesions produced was only 2 per leaf whereas inoculum from leaves produced 355 per leaf.

During growth of the embryo, the concentration of virus increased (Table 1). This was demonstrated also in another experiment in which 2 samples of embryos of different ages but of the same weight were compared. The extract from 22-day-old embryos produced 28 local lesions per leaf whereas the extract from 32-day-old embryos produced 90 local lesions. During growth of the embryo, there is an accumulation of nutrients which may be favorable for multiplication of the virus. It is also possible that virus movement may be associated with translocation of food and that increase of virus content in the embryo prior to maturation may be the result of movement of virus into it from other plant parts.

To indicate whether a similar change in virus activity occurs when immature seed are removed from plants and artificially dried, seed in the dough stage were harvested from systemically infected plants. For comparison of virus activity, several trifoliate leaves were collected from the same plants and stored at room temperature in sterile Petri dishes. At intervals, virus assays from seed coats, embryos, and leaves were made. After 5 days of storage, the seed coats were wrinkled and leathery; the leaves were yellow and brittle; and the cotyledons had changed from fleshy green to starchy white. After 1 week, the seed coats had become brown, a color not typical of normally ripened seeds. The relative virus concentrations in the different plant parts after different periods of storage are in Table 2.

It is evident from these data that a sudden inhibition of virus within the embryo of immature seed took place after 3–5 days of air-drying, when their comparable moisture contents were more or less similar to those of normally ripened seed. These results are in agreement with those recorded in Table 1. Other tests indicated that the dried seed coats of similar immature seed, stored for 10 weeks and similarly prepared for virus assay, contained sufficient virus to produce an average of about 10 lesions per leaf, making it appear that dehydration itself is not responsible for inhibition of virus in the embryo. Infected leaves and seed coats dehydrated to the same degree as the embryos did not show similar virus inhibition (Table 2). The virus of southern bean mosaic does not lose its infectivity in leaf tissue dried for several months.

Seed transmission.—The studies just reported show that the virus in the embryo is inhibited as the seed ripens. Completely ripened or dried seed do not trans-

TABLE 2.—*Effect of air-drying period on infectivity of southern bean mosaic virus in leaves and immature seeds of Logan bean as measured by the number of local lesions produced on primary leaves of Kentucky Wonder bean*

Storage period	Local lesions per leaf produced by inoculum from—: Seed coat sample (comparison 1)	Embryo sample (comparison 1)	Seed coat sample (comparison 2)	Leaf sample (comparison 2)	Embryo sample (comparison 3)	Leaf sample (comparison 3)
	Number	Number	Number	Number	Number	Number
None	1012	32	981	537	21	450
2 days	411	55	540	607	12	441
5 days	161	1	179	284	2	505
7 days	239	<1	163	738	0	521
14 days	73	0	37	270	0	440
21 days	25	0	29	575	0	429

mit the virus. The seed coats, though containing the virus for a much longer period than the embryo after seed maturation, have no direct connection with the developing seedling. It is only through mechanical means during germination that the virus in the seed coats could infect the seedling. However, if fresh immature seed from systemically infected plants are germinated immediately after harvest, the virus is transmitted to the germinating seedlings.

Seed-transmission tests were made with seed harvested from plants of several varieties of bean systemically infected with the virus. Mature seed were germinated in moist paper towels immediately after harvest and placed in crystallizing dishes. Immature seed that had attained full size, with fleshy soft seed coats, were removed from the pods aseptically and transferred into sterile Petri dishes fitted with filter papers and kept moist with sterile distilled water. After the seedlings had become 5–8 inches in length, the seed coats were removed and extracts of the seedlings prepared separately, then assayed. Totals of 50 mature and of 12–15 immature seed of each of the following systemically infected varieties were used: Black Valentine, Bountiful, Brittle Wax, Commodore, Dwarf Horticultural, Full Measure, Logan, Red Valentine, Small White, and State. In general, the seedlings derived from mature seed did not contain virus, but 5, 2, and 3 per cent of those of Logan, State and Brittle Wax showed southern bean mosaic, respectively. It was very possible that some not well-matured seed were used. Seedlings from immature seed showed a high percentage of infection. Infection for all varieties tested was 50 per cent or more, whereas for Logan it was 80 per cent.

To indicate whether the position of the ovule in the pod had any relation to virus infection, green pods containing at least 5 seed were collected from systemically infected plants of several varieties. The seed were remvoed from the pod in their proper order and extracts of the seed coat, cotyledon, and pumule of each seed were prepared separately and tested for virus. All seed within the pod transmitted the virus.

Throughout the literature, many other virus diseases of plants have been reported not to be seed-transmissible, even though virus was present during seed development. von Stelzner (12) indicated that potato viruses X and Y in the seed are inactivated during ripening, storage, and germination. A more important paper by Kausche (17) indicated further that the presence of a heat-stable substance extracted from ripened and germinated tobacco seeds inactivated tobacco mosaic virus. Raychaudhuri (11) also showed in his study on internal browning of tomato that the extracts of germinating tomato seed exerted an inhibitory effect on infectivity of tobacco mosaic virus in tomato. The following experiments were carried out in order to find out whether a similar substance extractable from bean seed exerted any inhibiting effect on southern bean mosaic virus.

Effect of juice of bean leaves on infectivity of southern bean mosaic virus.—It is known that the extract of leaves of certain plants inhibit some viruses in vitro (2,5,8). In this study crude juice of bean leaves added to southern bean mosaic virus prepared from bean and soybean leaves failed to cause inhibition. Juice from leaves of plants susceptible to either local or systemic infection reacted similarly.

Effect of extracts from bean seed on infectivity of southern bean mosaic virus.—The following procedure was used in preparing crude bean seed extracts. About 150 ml. of distilled water was added to 50 gm. of extracted juices of bean seed. The mixture was allowed to stand at room temperature for about 3 hours and later was frozen for approximately 16 hours. After being thawed, the suspension was strained through cheesecloth. The filtrate was adjusted to pH 5 and about 20 ml. of a 40 per cent solution of basic lead acetate was added to each 100 ml. of the mixture, until no further precipitation occurred. The mixture was centrifuged and the precipitate discarded. The excess lead was removed by the addition of a 20 per cent solution of $Na_2HPO_4 \cdot 12H_2O$. The final solution was concentrated in a 50°C. vacuum oven to about half its original volume. This was again centrifuged and the precipitate discarded. The final extract had a pH of about 6.

To test the inhibitory effect of this seed extract on southern bean mosaic virus, 0.1 ml. of virus inoculum was added to 10 ml. of extract. This mixture was used immediately to inoculate 1 primary leaf of each of 10 Kentucky Wonder bean seedlings. The opposite leaf of each seedling was inoculated with 10^{-2} dilution of the untreated virus, designated as standard inoculum. The virus to which the bean seed extract was added produced 95 per cent fewer local lesions than the untreated virus of the same dilution.

To determine whether a similar inhibitory material was present in immature and germinating seed, samples of healthy, fresh immature seed, air-dried immature seed, fully matured seed, and germinating mature seed from greenhouse-grown Logan bean plants were used for preparation of seed extracts. The procedure mentioned in the preceding paragraph was followed except for preparation of extracts of germinating mature seed. In this case, only cotyledons and unfolding plumules were used. Several tests were made; the results of one representative test are in Table 3.

Extracts from air-dried immature and mature seed caused greater inhibition of southern bean mosaic virus than extracts from fresh immature seed. Extracts from mature germinated seed caused even more inhibition.

Several dilutions of the mature seed extract were prepared by addition of a neutral phosphate buffer solution. To 10 ml. of each dilution of seed extract 0.1 ml. of virus inoculum was added. Virus activity of each of these solutions was compared with that of a standard virus inoculum of a dilution of 10^{-2} prepared from infected leaves. Primary leaves of Kentucky

TABLE 3.—*Effect of extracts of Logan bean seed of different stages of maturity or germination on infectivity of southern bean mosaic virus in standard (1–100) inoculum as measured by the number of local lesions produced on primary leaves of Kentucky Wonder bean*

Extract added to standard inoculum	Average local lesion per leaf in 10 replications	Reduction by extract
	No.	Per cent
None	638	
From fresh immature seeds	321	49.7
None	700	
From air-dried immature seeds	112	84.0
None	733	
From mature ungerminated seeds	27	96.2
None	784	
From germinating mature seeds	2	99.7

Wonder bean seedlings were inoculated with the various preparations and local lesions produced by each inoculum were counted. Diluting the seed extract gradually reduced its inhibiting effect on southern bean mosaic virus (Table 4).

Effect of diluting a mixture of virus and seed extract.—Twenty ml. of seed extract was mixed with 0.4 ml. of expressed juice of a plant infected with southern bean mosaic virus and allowed to stand for about 2 hours at room temperature. A series of dilutions of this mixture was made as in the previous experiment. Expressed juice from leaves containing southern bean mosaic virus was used in making a similar dilution series with distilled water.

Results of assaying the 2 series are in Table 5. The data indicate that southern bean mosaic virus is not inactivated by the bean seed extract but inhibited by it and that dilution reduces this inhibitory effect.

Instead of inhibiting the virus, the seed extract may decrease susceptibility of host cells to infection as suggested by Gupta and Price (6) in their study on virus inhibition by fungal growth products. To determine whether the dilution of the seed extract had an effect on susceptibility of the host cells, primary leaves of Kentucky Wonder seedlings were smeared with

TABLE 5.—*Effect of dilution of a mixture of seed extract plus juice containing southern bean mosaic virus on its infectivity on Kentucky Wonder bean plants*

Dilution of mixture	Local lesions per leaf produced by indicated inoculum	
	Diluted mixture	Standard inoculum from leaves
None	5	769
1:2	13	684
1:4	36	640
1:8	63	586
1:16	106	509
1:32	146	386
1:64	167	372

TABLE 4.—*Effect of dilutions of mature seed extracts on infectivity of southern bean mosaic virus in standard (1–100) inoculum as measured by the number of local lesions produced on primary leaves of Kentucky Wonder bean*

Dilution of extract to which standard inoculum was added	Average local lesion per leaf in 10 replications	Reduction by extract
	No.	Per cent
Standard inoculum only	202	94.5
Undiluted extract	11	
Standard inoculum only	298	85.2
1:2 dilution	44	
Standard inoculum only	280	68.2
1:4 dilution	89	
Standard inoculum only	200	48.0
1:8 dilution	104	
Standard inoculum only	278	44.9
1:16 dilution	153	

extracts from seed and washed immediately as well as 15, 30, 40, and 60 minutes later. These leaves were then inoculated with 1:100 dilution of southern bean mosaic virus. The opposite leaves of each seedling so treated were smeared with a buffer solution, washed in the same manner as were those smeared with the seed extract and inoculated with virus of the same dilution. Data in Table 6 indicate that the seed extract apparently had no effect on the host cells since the numbers of local lesions produced on the treated leaves were about the same as on leaves treated with buffer solution.

Nature of inhibiting substance in seed extract.—An extract from the seed, heated in a water bath for 90 minutes at 100°C., still maintained its inhibiting effect on southern bean mosaic virus. When concentrated HCl was added to the extract and the mixture was heated as just described, the extract jelled. After being neutralized, it again liquified; the inhibiting material was still present. When sodium hydroxide was added to the seed extract, the mixture turned brown; when neutralized, the mixture was capable of inhibiting the virus. It is apparent from these tests that the inhibiting substance in mature bean seed is heat-stable and resistant to both acid and alkali.

DISCUSSION.—In 1915 Allard (1) indicated the possible association of tobacco mosaic virus and the development of seed. He traced the distribution of tobacco mosaic virus in infected tobacco plants and

TABLE 6.—*Relation of infectivity of southern bean mosaic virus to time of contact of seed extract with subsequently inoculated leaves of Kentucky Wonder bean*

Period of contact (minutes)	Local lesions per leaf in 10 replications on leaves previously smeared with—	
	Seed extract	Buffer solution
0	319	284
15	323	353
30	197	180
40	200	204
60	272	296

found that it was distributed through the placental structures of the seed, reaching even the ovules. Since tobacco mosaic is not known to occur as a result of embryonic transmission of virus, he assumed that a very efficient barrier guarded against infection of the embryo or the subsequent successful continuation of the disease from parent to seedling.

Duggar (4) studied the relation of seed transmission of tobacco mosaic virus and indicated that the non-transmissibility of tobacco mosaic virus through seed might be due to adsorption and inactivation through storage proteins.

Bennett and Esau (3), in their study on curly top virus in sugar beet and tobacco, found that a high virus concentration is reached in the matured seed of infected plants. The virus, however, was found most abundant in the vascular region of the seed although none was recovered from the embryo. Bennett and Esau indicated that the embryo of the infected seed developing into a seedling is able to drain the seed of stored food without acquiring any of the virus.

In the study herein reported it is also shown that the virus of southern bean mosaic was present in all flower and fruit parts of infected plants, including the embryo. During the process of seed maturation there appeared to be a gradual increase in virus concentration in the embryo. During the same period, however, the concentration in the seed coat and pod decreased. As dehydration of the seed started, either naturally or artificially, the virus activity in the embryo immediately ceased. This did not occur in the seed coats or leaves. Through further seed transmission tests, it was also demonstrated that immature seed from systemically infected bean, when germinated in aseptic condition, showed a high percentage of virus transmission, whereas in case of well-mature seed the virus transmission was nil. It is possible that some chemical inhibition of southern bean mosaic virus in the embryo took place during the final stage of maturation. This might be due to certain chemical changes or to the action of an enzyme or some other chemical substance produced during the dehydration process of ripening.

The presence of a southern bean mosaic virus inhibitor in bean seed extract strongly implies that the chemical substance produced during the dehydration process of ripening serves as an inhibitor which makes the virus inactive during germination and seedling development. Since such an inhibitor is present in immature bean seed it is conceivable that the inhibitor increases in amount during seed ripening and seed germination. However, the fact that the virus concentration increases in the embryo during seed development, with a maximum just prior to the dehydration stage, does not entirely support the theory that the inhibitor present in the immature seed is responsible for the complete inhibition of virus in mature seed. The demonstration of inhibition in vitro by bean seed extract may possibly be unrelated to the fate of the virus in the seed. Pound and Cheo (9) found that the inhibitor of cucumber mosaic virus 1 present in all spinach plants did not inhibit the virus in Nobel spinach plants themselves when the juices were extracted. It did, however, inhibit the same virus in inoculum from some other host. This would indicate that an inhibitor may act against a virus in vitro but have little effect on it while in the plant.

Protein synthesis within the developing seed continues during the dehydration process of the final stage of seed maturation. Formation of protein is a condensation process which accompanies desiccation of the seed. The decrease in water content would, of course, favor the synthetic action of whatever proteinases were present. On desiccation, amino acids and most of the asparagine disappear and protein increases in the storage cells. Since virus particles are protein in nature, the process of protein synthesis in seed might possibly have some inhibiting effect on the virus.

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THE RELATION OF LIGHT TO THE PRODUCTION OF PYCNIDIA BY PHYSALOSPORA OBTUSA[1]

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SUMMARY

Cultures of *Physalospora obtusa* grown on potato-dextrose agar did not ordinarily produce pycnidia in the absence of light. By irradiating cultures with fluorescent light of different intensities and qualities, mature pycnidia were produced. Both monoconidial and monoascosporic isolates, in irradiated cultures, produced abundant pycnidia. Since ascospores could not be produced in culture, monoconidial isolates were used for most tests.

Irradiation of cultures for 12–18 hours at approximately 200 foot-candles was sufficient to induce pycnidial production. Conversely, an intensity of approximately 20 foot-candles for 100 hours was also sufficient to induce pycnidial production.

The quality of light also affected pycnidial production. The blue range of the visible spectrum was apparently most effective. However, ultra violet radiations apparently had no effect on the process.

Irradiation of a restricted part of a culture stimulated production of pycnidia. The stimulus apparently resulting, from the irradiation, was not translocated to all parts of the same culture. Pycnidia were only produced in the areas that were directly and indirectly irradiated and were generally most abundant in areas that were directly irradiated.

In the absence of light, cultures exposed to a wide range of temperatures did not produce pycnidia. With continuous irradiation at adequate intensities they were produced at 10°C., but not at 5°C.

The fungus, *Physalospora obtusa* (Schw.) Cooke, which incites the important black rot and leaf spot diseases of apple, has been studied extensively but many of the factors affecting its growth and reproduction are still unknown. Several workers experienced some difficulty in obtaining mature pycnidia in culture. Foster (4) found that, on potato-dextrose agar, pycnidial production occurred within 8–16 days whereas Hesler (5) obtained mature pycnidia from either monoconidial or monoascosporic cultures in about 10 days. Ayers (2) used the production of mature conidia in monoascosporic cultures as a means of proving the relationship of the ascigerous stage of this fungus. In contrast, Brooks and Cooley (3) used mycelial inoculum in their tests because *Sphaeropsis malorum* did not fruit readily in their cultures. Though Stevens and Wilcox (9) were able to obtain pycnidia in pure culture, they stated that many investigators rarely found these pycnidia in culture. Reporting on the cultural behavior of *S. malorum*, Mohendra and Mitra (7) concluded that it was a fungus which gradually lost its sporulating capacity under cultural conditions, even when sporal inocula were used for each transfer.

Investigation of the factors affecting asexual sporulation of *P. obtusa* has been limited. Shear and Stevens (8), in obtaining pycnidia in culture, reported that the factors concerned with sporulation were believed to be temperature and/or humidity or some unrecognized factor. The need of light to induce pycnidial formation was indicated by the work of Stevens and Wilcox (9) and Aycock (1). Similarly, Foster (4) was able to obtain pycnidia by alternate exposure to diffuse light.

This paper is a report of an evaluation of some of the effects of various intensities and qualities of light on the mycelial and pycnidial development of *P. obtusa* in pure culture.

MATERIALS AND METHODS.—Several monoconidial and monoascosporic isolates of *P. obtusa* from various localities in North Carolina were used. Regardless of the source of cultures, those in any one test were grown in Petri dishes and were obtained from a single isolate which had been cultured on potato-dextrose agar in the absence of light. The test cultures in covered Petri dishes were placed singly on the bottom of an open-topped box 12 in. deep. Over the top of this box was fitted a 2-tube, 40-watt, daylight fluorescent light fixture of the commercial type. The light intensity at the surface of each irradiated culture was measured in foot-candles (F.C.) with a Weston Illumination Meter, equipped with a quartz filter. The cultures were irradiated at room temperatures of approximately 25–30°C. The cultures used as checks were placed in light-proof metal containers immediately after inoculation, but in other respects were kept under the same conditions as the test cultures.

Most of the tests were terminated within 6 days, since preliminary trials had shown that pycnidia would mature in less than this time. This eliminated the difficulty of making detailed observations of cultures which would generally become heavily pigmented if grown for more than 6 days (Fig. 1, D).

Results of the tests were determined in terms of the number of pycnidia produced per culture. These pycnidia were sampled to determine the stage of development of the conidia.

EXPERIMENTAL RESULTS. — *Pycnidial development under continuous irradiation.*—In a preliminary test, 7

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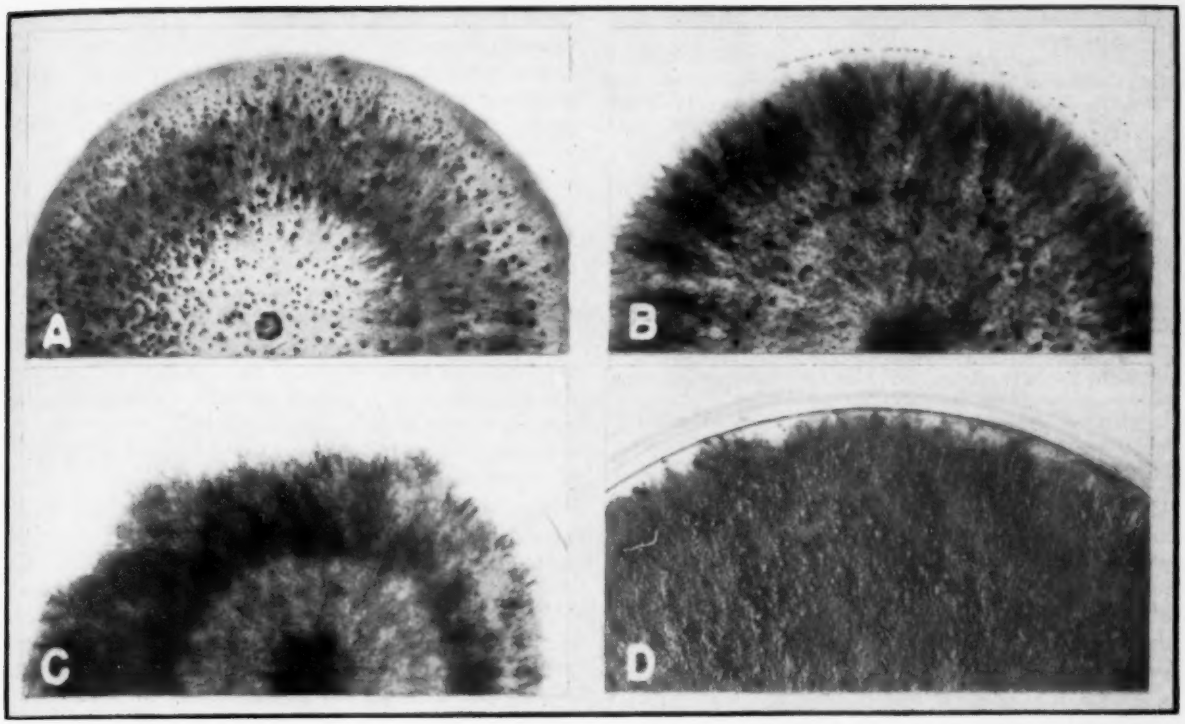

FIG. 1. Pycnidial production by cultures of *Physalospora obtusa* on potato dextrose agar. A) Abundant pycnidia after 116 hours of irradiation with fluorescent light at 250 foot-candles intensity. B) Moderate number of pycnidia after 116 hours of irradiation at 52 foot-candles. C) No pycnidia after 116 hours in dark in absence of light. D) Portion of a 20-day-old irradiated culture showing heavy pigmentation and pycnidia visible at the surface.

cultures were exposed to light at an intensity of 237–277 foot-candles for 149 hours. All irradiated cultures produced an abundance of pycnidia, but non-irradiated cultures produced none. The mycelial mats produced during irradiation were distinctly darker than those produced in absence of light.

Similar results were obtained when the test was repeated using 25 monoconidial isolates and 16 monoascosporic isolates. The monoascosporic isolates were originally obtained from the same perithecium. In this test, there was some variation in abundance of pycnidia among different isolates and in pigmentation of mycelial mats. Similar variations occurred in other tests involving both monoascoporic and monoconidial isolates. Since there was no consistent difference in the response of the various monoascosporic and monoconidial isolates, subtransfers from a single monoconidial isolate were used in most of the subsequent tests. This isolate was maintained in the absence of light through 17 mycelial transfers for more than 4 months. Throughout this period there was no indication of reduction in sporulation in irradiated subtransfers of this isolate.

Pycnidial development under discontinuous irradiation.—To determine the effect of length of the irradiation period on production of pycnidia, 12 cultures, plus additional ones for checks, were used in each of 4 separate tests. Intensities ranging from 209–292 foot-candles were used in these tests since it had been determined earlier that a light intensity of 200 foot-candles was sufficient to induce abundant pycnidial formation and an intensity of 800 foot-candles was not inhibitory. Prior to irradiation, each culture was grown in darkness until the fungal mat was approximately 60 mm. in diameter. After irradiation, each culture was placed in a light-proof metal can and left inside the light chamber. Thus, all cultures of any one test were kept at approximately the same conditions of temperature and moisture throughout the test. Irradiation periods in these tests ranged from 1–108 hours.

Since light intensity was measured separately for each Petri-plate culture, the product, foot-candles $\times$ hours, was used to express light treatment. In these tests the threshold for pycnidial production occurred at about 3,000 foot-candle-hours (Table 1). At intensities of 209–292 foot-candles, 12–18 hours was the minimum exposure period for production of an appreciable number of pycnidia.

Pycnidial development in irradiated mycelium.—Examination of cultures in previous tests indicated that pycnidia were produced continuously by irradiated, actively growing mycelia. The initiation and maturation of pycnidia were studied by growing cultures under light continuously for 79 and 107 hours follow-

TABLE 1.—*Pycnidial development under discontinuous irradiation*

Range of irradiation periods in hours[a]	Average foot-candle-hours[b]	Average number of pycnidia per culture[c]
1–3	388	1.0
3–5	872	0.4
6–8	1549	0.2
8–10	2172	2.4
8–11	2383	1.6
10–13	2920	5.4
14–18	4518	21.6
35	8743	275.0
35–108	14054	300.0

[a] Five cultures were used for each range of irradiation.
[b] Light intensity was measured for each culture. The range of average intensities was 209–292 foot-candles.
[c] Of 38 check cultures, included in this test, 31 produced no pycnidia; 5 produced 1 each; 1 produced 2; and 1 produced 4.

ing inoculation. After irradiation a strip of agar 4 mm. wide was cut radially from each culture. These strips were removed, cut into 10 equal cross sections, and examined microscopically to determine the stage of development of the majority of pycnidia in each section.

As the fungus grew over the medium the first sign of a pycnidium was a small cluster of short hyphae. In slightly older portions of the cultures immature pycnidia with distinct walls were formed. In still older portions the pycnidia contained hyaline conidia still attached to the conidiophores. In the oldest portions of the cultures, pycnidia contained mature, tan-colored conidia. The rate at which these changes took place was followed for the 2 time intervals. For example, in cultures irradiated for 79 hours the first 6 sections from the center, i.e., the oldest sections, contained pycnidia with definitely formed walls. The remaining 4 sections contained pycnidial initials in a bunched-mycelial stage. With 107 hours of irradiation the first 4 sections from the center contained pycnidia with mature, tan-colored conidia.

Pycnidial development under different light intensities.—To determine the minimum light intensity required to initiate formation of pycnidia, cultures in 4 tests were irradiated at various intensities below 75 foot-candles for periods of time greater than 90 hours. In these tests the desired light intensity was obtained by placing sheets of uniform white paper over the tops of light-proof containers. After inoculation, Petri-dish cultures were placed within these containers and the intensity of light impinging on each culture was measured at the surface of the dish. In these tests the threshold of light intensity for pycnidial production was about 20 foot-candles.

In test 1, at an intensity of 8.8 foot-candles or less, no pycnidia were produced, whereas in test 3 few pycnidia were produced at intensities of less than 15 foot-candles. Few pycnidia were produced at less than 20 foot-candles in tests 2 and 4 although considerable variation in pycnidial production was evident in both tests. In the check cultures for 3 of these tests, no pycnidia were formed. In the 8 check cultures in the fourth test, a total of 6 pycnidia were produced. The relative abundance of pycnidia produced in cultures grown at high intensity, at low intensity, and under check conditions is shown in Figure 1.

Pycnidial development under alternate periods of light.—The effect of alternate periods of light on the production of pycnidia was studied by 2 methods. The first method consisted of irradiating a set of cultures continuously for 86 hours at 230 foot-candles followed by a period of 152 hours of darkness but otherwise under comparable conditions. By this method it was found that pycnidia were produced only in that part of the culture which had been irradiated. The irradiated mycelium was deeply pigmented and all mycelia in irradiated cultures were more deeply pigmented than in check cultures.

The second method consisted of dividing into short periods the known time required, under continuous irradiation, to induce pycnidial formation. In this test, different sets of cultures were irradiated at 200 and 282 foot-candles for 19 hours, 16 hours, and 4 4-hour periods alternating with 3-hour dark periods. In this test irradiation for 4 4-hour periods alternating with 3-hour dark periods was as effective as the continuous 16-hour period in inducing pycnidial formation. Neither of these exposures was as effective as continuous irradiation for 19 hours.

Pycnidial production in relation to temperature and light.—The effect of temperature on production of pycnidia was studied, as earlier work on other fungi (6) indicated that this factor might replace the effect of light. Cultures were grown at 8 temperatures ranging from 8°C.–36°C. in the absence of light. No pycnidia were produced at any of the temperatures used, although the organism grew readily over this wide range of temperatures.

To study the combined effect of temperature and light on production of pycnidia, sets of cultures were subjected to 4 different treatments immediately after inoculation. In the first treatment, cultures were exposed at 27–32°C. to continuous irradiation. All cultures of this set produced a moderate number of pycnidia. In the second treatment, cultures were irradiated at 27–32°C. for 2 periods of 13 hours and 11 hours, alternating with 2 periods of 12 hours and 14 hours at 8°C. in the absence of light. Again, all cultures produced pycnidia but distinctly fewer pycnidia than in the first treatment. None of the cultures of these 2 treatments exhibited any noticeable difference in general growth characteristics. In the third and fourth treatments, which were replicates of the first 2 except that they were carried out in the absence of light, no pycnidia were produced.

In another test, several cultures approximately 40 mm. in diameter were irradiated continuously for 163 hours under an average intensity of 515 foot-candles at a temperature of 5°–6°C. The same irradiation period was used for other cultures approximately 50

mm. in diameter exposed to an average intensity of 570 foot-candles at 11°–13°C. No pycnidia were produced in the cultures irradiated at the lower temperature and none were found a week after termination of the test period. Abundant pycnidia were produced in the new growth of cultures irradiated at the higher temperatures, and some were found to contain nearly mature conidia.

After these examinations were completed, the cultures irradiated at 11°–13°C. were divided into groups and treated as follows: 1) placed in absence of light at room temperatures; 2) irradiated at 10°C.; 3) irradiated at room temperature; 4) placed under check conditions at 12°C. These cultures were maintained under such conditions until the mycelia of most of the cultures had completely covered the medium. On examination it was found that very few additional pycnidia were produced in the central region of any of the cultures regardless of post treatments; however, pycnidia were abundant in the outer regions of the cultures, these regions having developed during the irradiation. The post treatments apparently had little or no effect upon the pycnidial production.

Pycnidial development under different qualities of light.—To determine the effect of ultra violet light upon pycnidial production of *P. obtusa*, Petri-dish cultures with lids removed were irradiated at several intensities for periods ranging from 2 seconds to 26 minutes. These cultures were then placed in darkness to allow sufficient time for pycnidial production. No pycnidia were found in any of the cultures of these tests, but the cultures exposed for the longer periods showed some suppression in growth of the aerial mycelia.

The effect of light of other qualities on the production of pycnidia was studied. Filters were used to transmit light in the red, blue, green, yellow, and white ranges. The fluorescent light source was adjusted in each case so that comparable intensities ranging from 15–25 foot-candles were transmitted for periods of 119–170 hours. Results from 2 of the tests illustrate the general response to these treatments. No pycnidia were produced in cultures irradiated under the transmitted red light, very few under the yellow, and a moderate number under the green or white light. All cultures irradiated under the transmitted blue light produced abundant pycnidia.

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PERINEAL PATTERNS OF ROOT-KNOT NEMATODES [1]

A. L. Taylor, V. H. Dropkin[2] and G. C. Martin[3]

SUMMARY

Females of each species and subspecies of the genus Meloidogyne (root-knot nematodes) have perineal patterns which are variations of a typical pattern distinct from those of the other forms. Typical patterns can be recognized, and being more common than extreme variants, are of value in identification of populations.

Although root-knot nematodes have long been recognized as important parasites of many crop and other plants, no detailed descriptions of their morphology were published until 1949, and they were grouped under a single species name, *Heterodera marioni* (Cornu, 1879) Goodey, 1932. At that time, Chitwood (2) transferred them to the genus *Meloidogyne* Göldi, 1887, describing 5 species and 1 subspecies. In 1952 (3), he described an additional subspecies. Another species was described by Loos in 1953 (5).

Among the characters used in differentiation of the species is the "fingerprint-like" pattern formed by the cuticular striae of the perineal region of the adult female. The perineal pattern of each species was illustrated in Chitwood's original paper (2) by a photograph and drawings for each species described. The second paper (3) was not illustrated, but contains a reference to a drawing in the first paper. The description by Loos (5) contains a single drawing of the perineal pattern. Sasser (6) presented excellent photographic illustrations of some of the variations in the perineal pattern of 5 of the known forms.

The authors have studied perineal patterns of specimens obtained from preserved plant material used by Chitwood in preparation of his first paper, as well as specimens obtained from many locations in the United States and foreign countries. One of us (4) has made detailed studies of inheritance of various features of the perineal pattern in cultures started with a single larva or from single egg masses and maintained by similar transfers through several generations. In the course of our work, much information on variation in perineal patterns has been accumulated, numerous photographs of perineal patterns have been made, and techniques for mounting perineal patterns for examination have been developed. The purpose of the present paper is to supplement previous literature by photographs and descriptions of the most common variations of the perineal patterns of all the described forms, to present a key designed to facilitate their identification, to suggest a system of nomenclature for the perineal pattern, and to describe mounting techniques.

TECHNIQUES.—Root-knot nematode patterns for examination can be obtained from either fresh or preserved plant material; the exact method of preparation depends on the kind and amount of material available. Infected plant material may be preserved in 5 per cent formaldehyde until required. Apparently, if the material is in good condition when preserved, it remains usable for many years. Care must be taken to avoid drying or other deterioration prior to preservation.

With material preserved in formaldehyde for 24 hours or longer, preparation of perineal patterns for examination starts with dissection of the adult females from the plant tissue. This is done with the aid of a binocular dissecting microscope at magnifications of 10× to 30×, the plant material being in water in a Syracuse watch glass or other shallow container. When the nematode has been dissected out of the plant tissue, the sharp point of a small scalpel[4] is

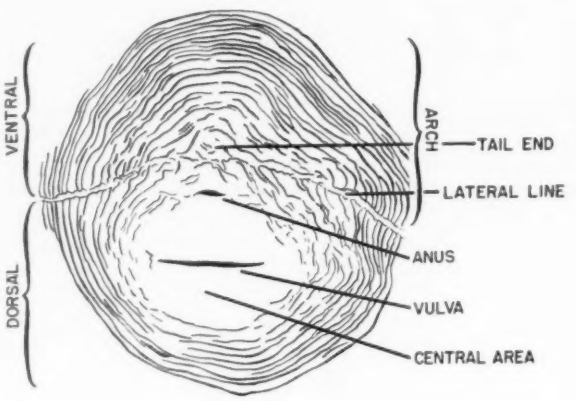

FIG. 1. Perineal pattern of *Meloidogyne javanica* to illustrate suggested nomenclature. This figure was prepared from the same photograph as Fig. 2, A.

[1] Accepted for publication September 8, 1954.

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[3] Nematologist, Rhodesian Tobacco Association, Rusape, Southern Rhodesia.

[4] For this purpose, the small scalpels used in eye surgery are ideal. Lacking this, a sharp dissecting needle with a spear point, a needle ground flat and sharpened, or a piece broken from a razor blade may be used.

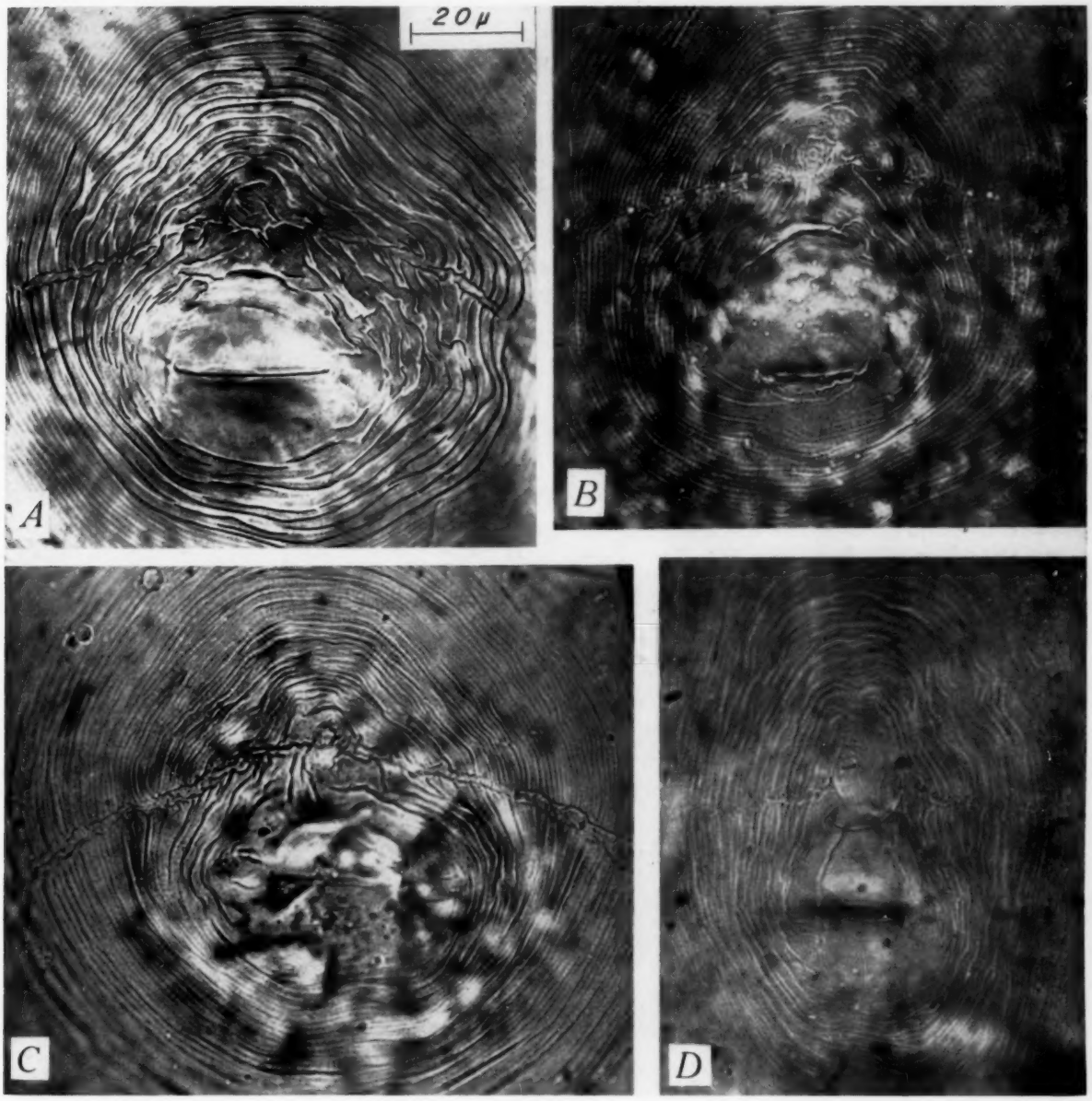

FIG. 2. Perineal patterns of *Meloidogyne javanica*. A. Nematology Collection V2-8 (Population 11C). B and C. Greenhouse culture. D. Nematology Collection X-14 (Topotype).

used to puncture the body at a point slightly posterior to its widest part. The specimen is then lifted on the point of the knife and transferred to a dry microscope slide, which is substituted for the Syracuse watch glass on the microscope stage. The puncture is enlarged to a cut, which severs the nematode body at right angles to its long axis. A dissecting needle is used to steady the nematode during this operation. The more or less hemispherical posterior portion of the body is placed with the cut surface in contact with the slide. If the anterior portion of the body is to be examined also, it is oriented so that the neck is visible. A cover glass is gently lowered over the specimen and a drop of lactophenol solution[5] placed to flow between the cover glass and the slide. With any excess lactophenol blotted up, the slide is ready for examination, though it may be sealed if desired.

Perineal patterns may be obtained from heavily infected fresh roots by use of the Waring Blendor. About 2 gm. of small roots are cut in small pieces and placed in the blender with about 80 ml. of water. The

[5] Lactophenol solution is made by mixing 3 parts melted phenol, 1 part of lactic acid, 2 parts of glycerine, and 1 part of water.

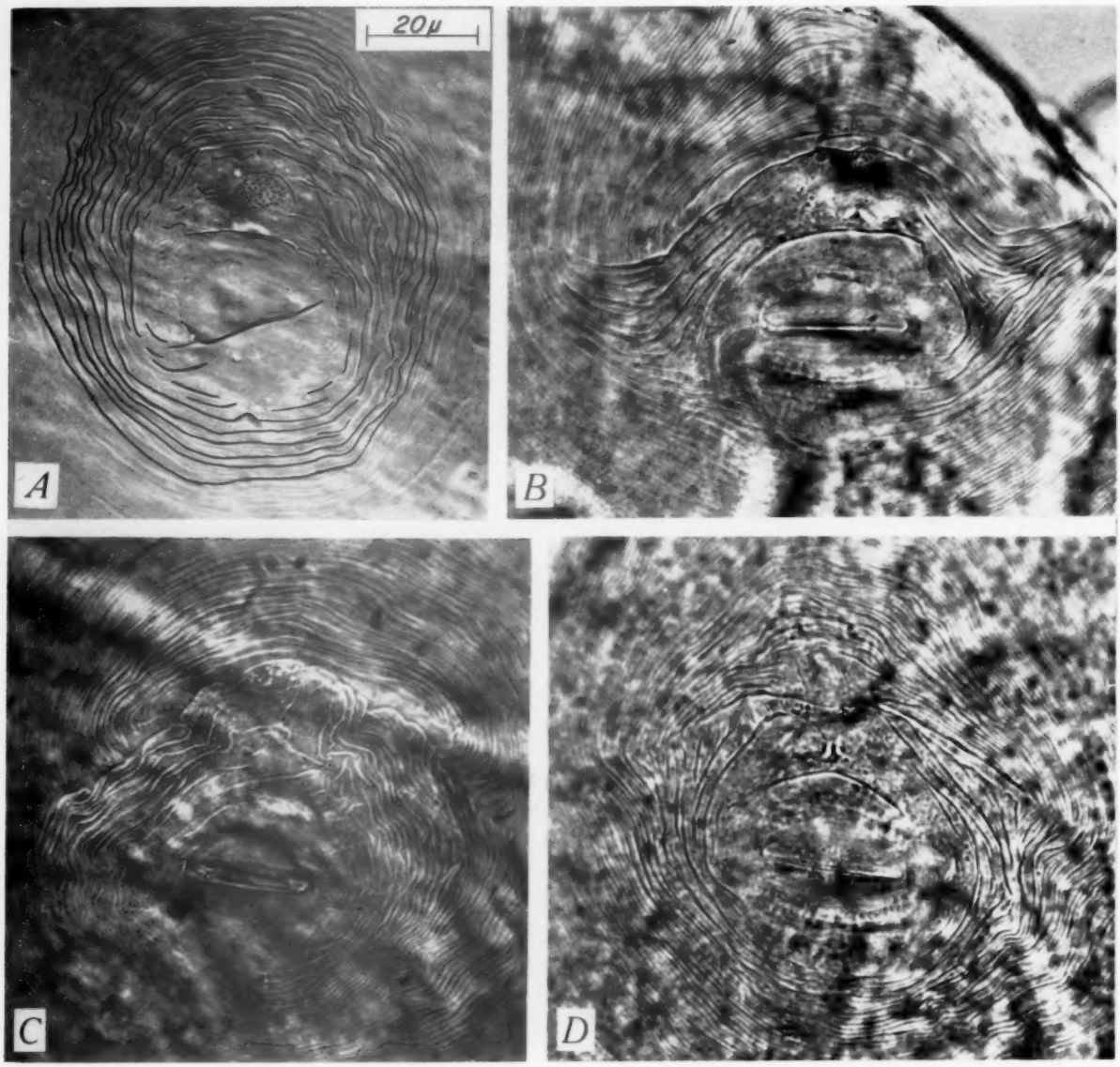

FIG. 3. Perineal patterns of *Meloidogyne hapla*. A, B and D. Greenhouse culture. C. Nematology Collection X-11 (Syntype).

blender is then operated for about 1 minute and emptied onto a 60-mesh sieve (openings 0.250 mm.) over a 200-mesh sieve (openings 0.074 mm.). The material on the sieves is washed with a stream of tap water. The residue on the 200-mesh sieve is washed into a beaker by inverting the sieve and pouring a small quantity of water through it. This residue will contain pieces of cuticle of females which have been cut by the knives of the blender. These can be mounted immediately or stored indefinitely in 5 per cent formaldehyde. The pieces of cuticle containing the pattern can usually be distinguished, even under the dissecting microscope, since a rounded thickened area is visible. Mounting is a simple matter of transferring these to a drop of lactophenol solution on a microscope slide. For routine examination, no particular care need be taken in orientation since the pattern can be seen from either side of the transparent cuticle. If mounted with the pattern on the upper surface, such specimens are particularly favorable for photographing.

Another method of preparing temporary mounts of perineal patterns from fresh material is to place the nematode in a small drop of water on a slide. A cover is placed over the specimen and the slide examined under a microscope. By moving the cover glass, the specimen is rolled until the neck is just under the body, then flattened by pressure on the cover glass. If properly done, this places the perineal pattern in

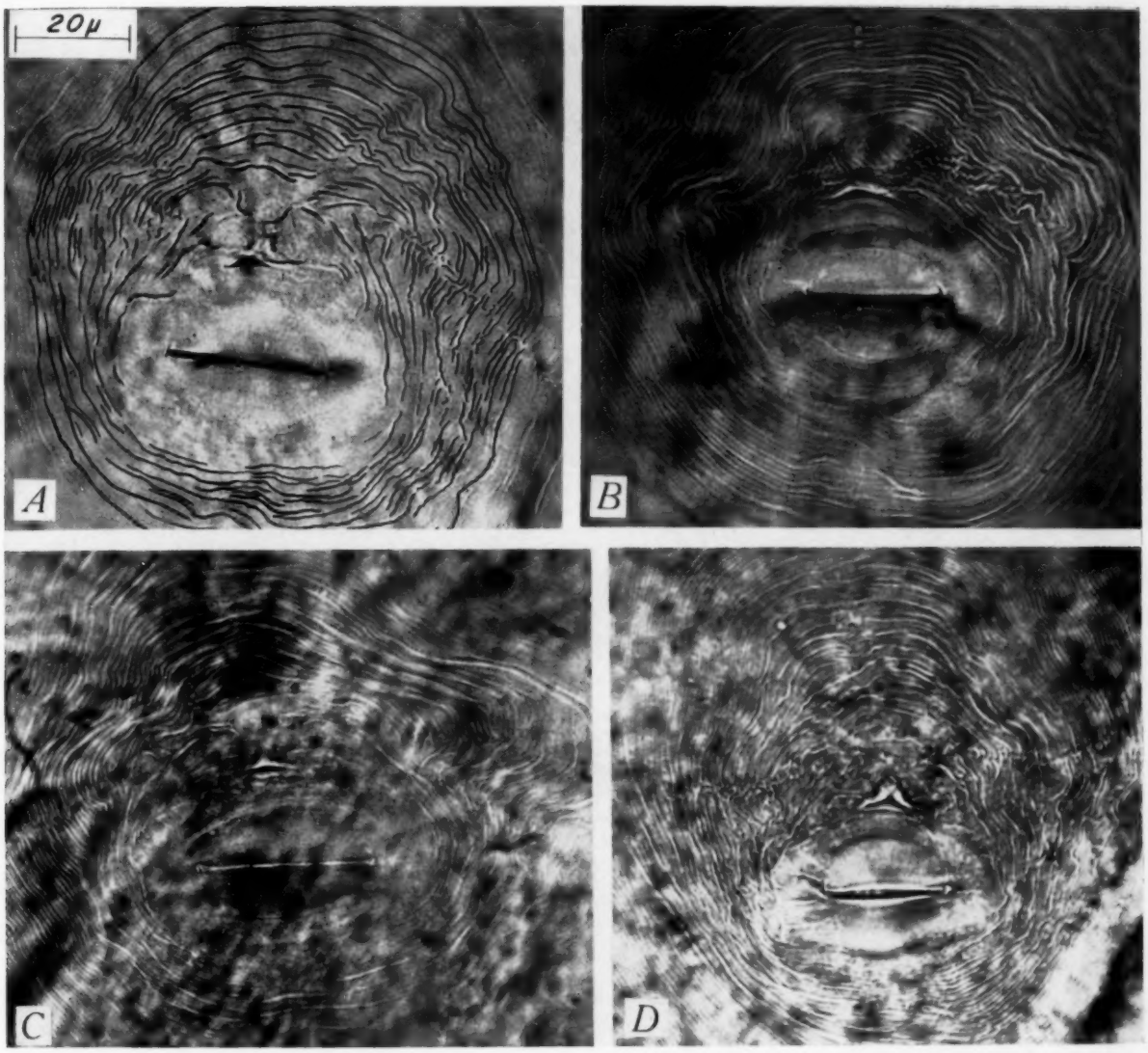

FIG. 4. Perineal patterns of *Meloidogyne arenaria*. A, B and D. Greenhouse culture. C. Nematology Collection V2-4 (Syntype).

position for examination, though occasional specimens will be damaged as the body bursts when flattened.

For critical examination of perineal patterns, the oil immersion objective of the microscope is used. The photomicrographs in this paper were made at a magnification of about 750× on Eastman Contrast Process Panchromatic film, following standard procedures. The drawing illustrating the nomenclature of the perineal pattern (Fig. 1) was made from an enlargement of the photograph of the pattern shown in Fig. 2, A, by inking in the desired lines with waterproof ink and bleaching the photograph. Ink was also used to emphasize certain features of the first of the series of photographs of perineal patterns of each species.

NOMENCLATURE OF THE PERINEAL PATTERN. — A drawing of the perineal pattern of *Meloidogyne javanica* (Treub, 1885) Chitwood 1949, is shown in Figure 1. In this illustration, as in the other illustrations of this paper, the perineal pattern is shown with the vulva more or less horizontal and below the anus. The upper portion of the drawing represents the morphologically dorsal sector of the pattern and the lower portion the ventral sector. The 2 sectors are separated by lateral lines, which are formed in this species by a series of distinct discontinuities in the striae. These were referred to as incisures by Chitwood (2). Lateral lines may be seen in the illustrations of the other species as a series of irregularities in the striae, or the dorsal and ventral striae may meet at an angle along the lateral lines. The point at which the

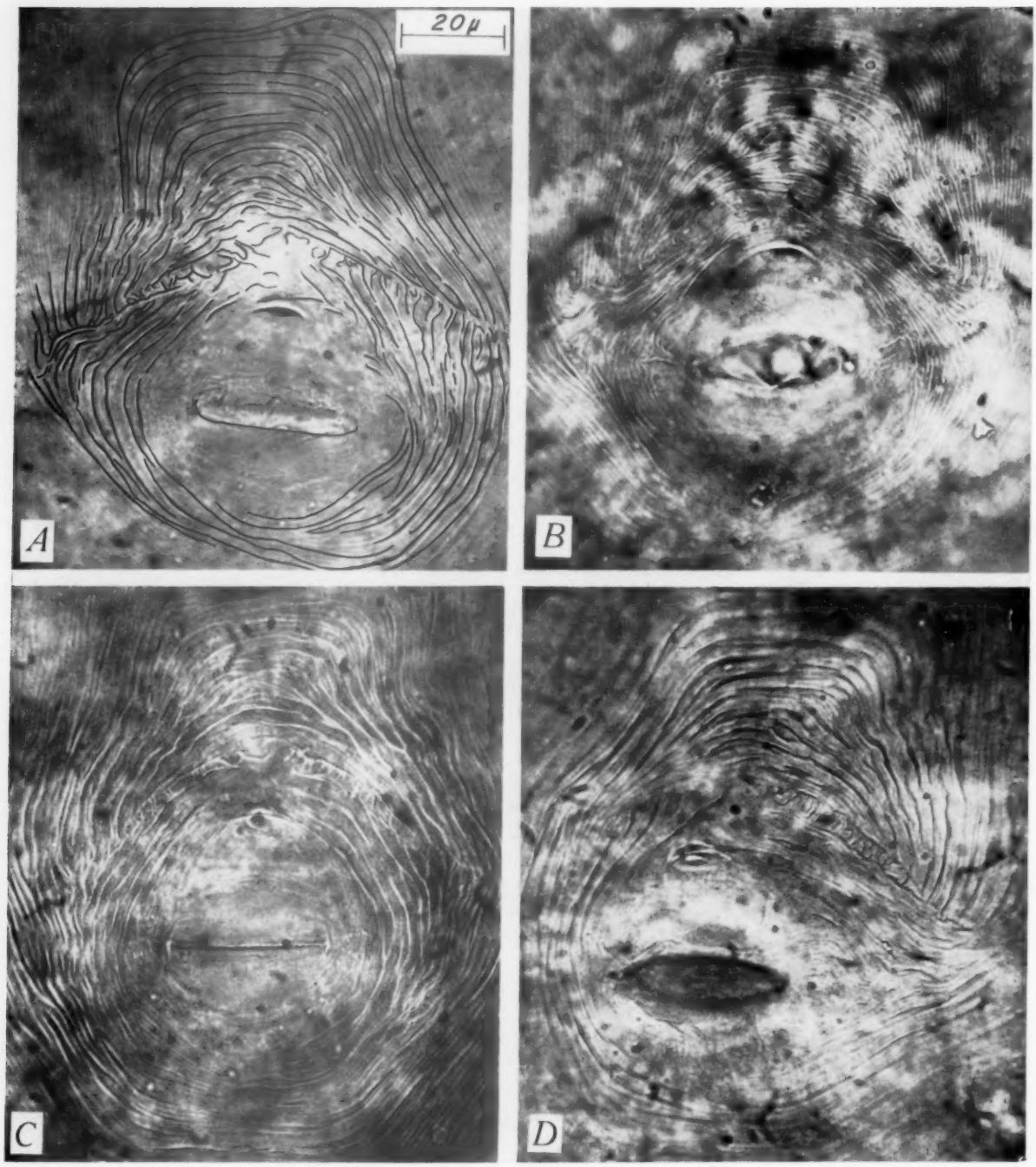

FIG. 5. Perineal patterns of *Meloidogyne arenaria thamesi*. A, B, C and D. Nematology Collection V4-9 (Topotypes).

2 lateral lines meet just above the anus is the tail tip. The striae of the dorsal sector of the pattern form the arch. In the ventral sector, the striae surround the central area, which includes the vulva and anus and may also include a few irregular striae. The pattern may be divided into left and right sides[6] by an imaginary line passing through the middle of the

[6] In this paper, reference is made to right and left sides of the pattern as they appear with respect to the observer in the illustrations or under the compound microscope.

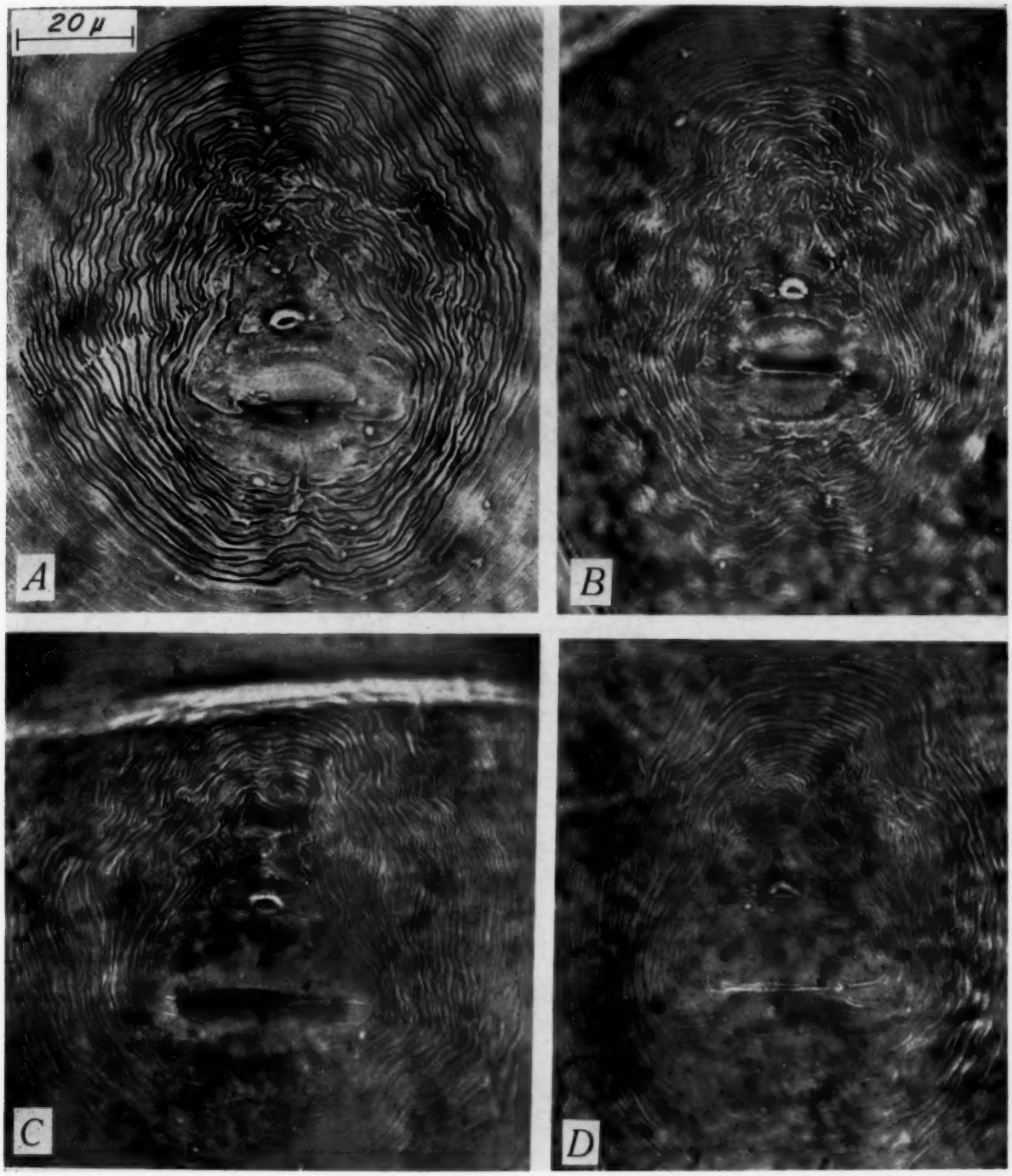

FIG. 6. Perineal patterns of *Meloidogyne incognita*. A and B. Greenhouse culture. C and D. Nematology Collection V4-24 (Population 11D).

vulva and the tail tip. Perineal patterns are often asymmetrical with respect to this line. Striae are sometimes extended on one or both sides of the pattern forming "wings" (figs. 3, B, 4, C, and 5, D).

DESCRIPTIONS OF THE PERINEAL PATTERNS.—It is probable that no 2 perineal patterns are exactly alike. However, each species has a typical pattern distinct from those of the other species. Within the species, the patterns vary to a greater or lesser extent so that some of the extremes in one species may be mistaken

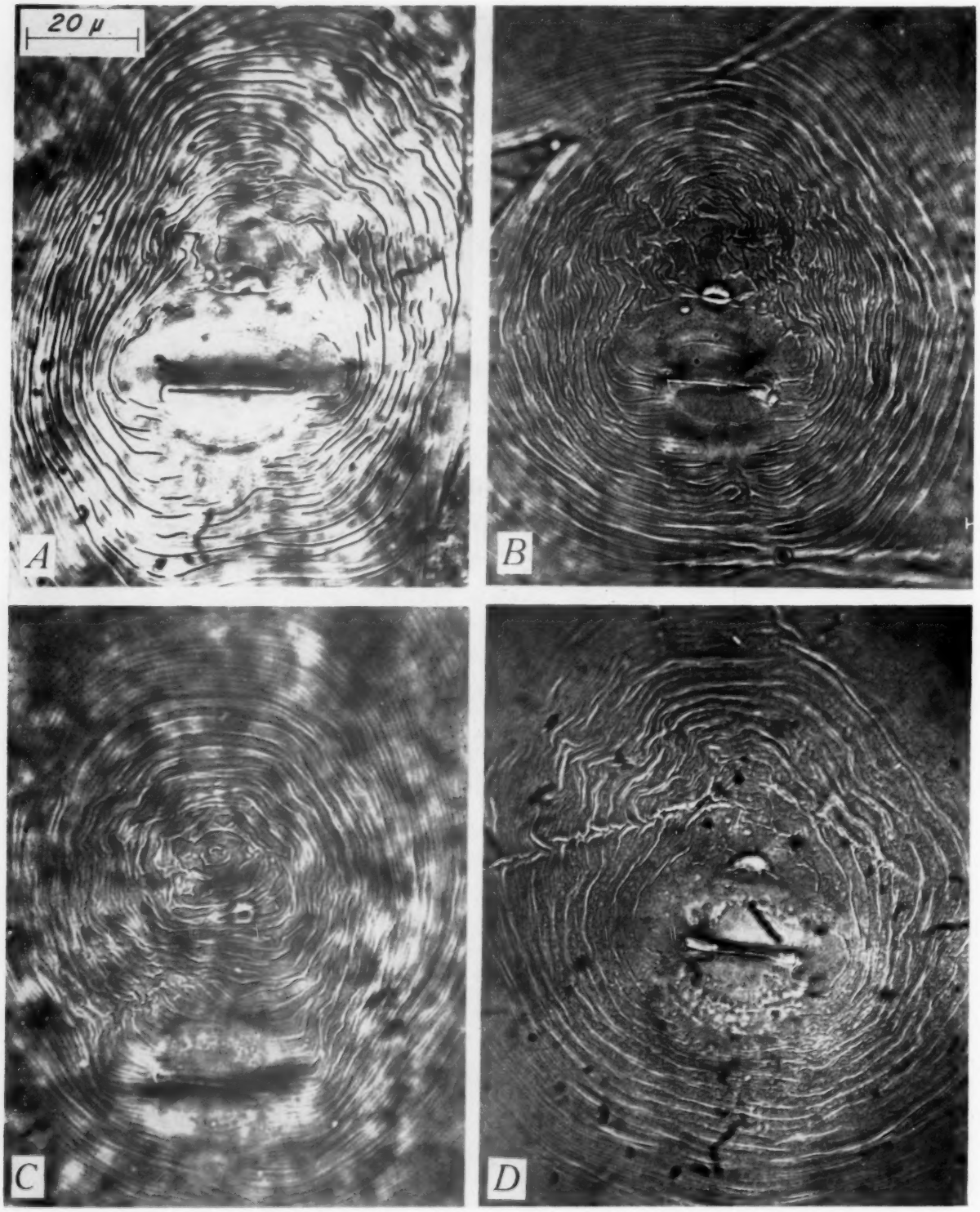

FIG. 7. Perineal patterns of *Meloidogyne incognita acrita*. A. Nematology Collection X-15 (Syntype). B and C. Greenhouse culture. D. 11th generation pure line (Dropkin, 1953). Note the fold along the left lateral line which might be mistaken for an incisure.

for those of another species, as recognized by Allen (1). However, such variants are much less common than the typical patterns and the typical patterns can always be found if an adequate sample of the popula-

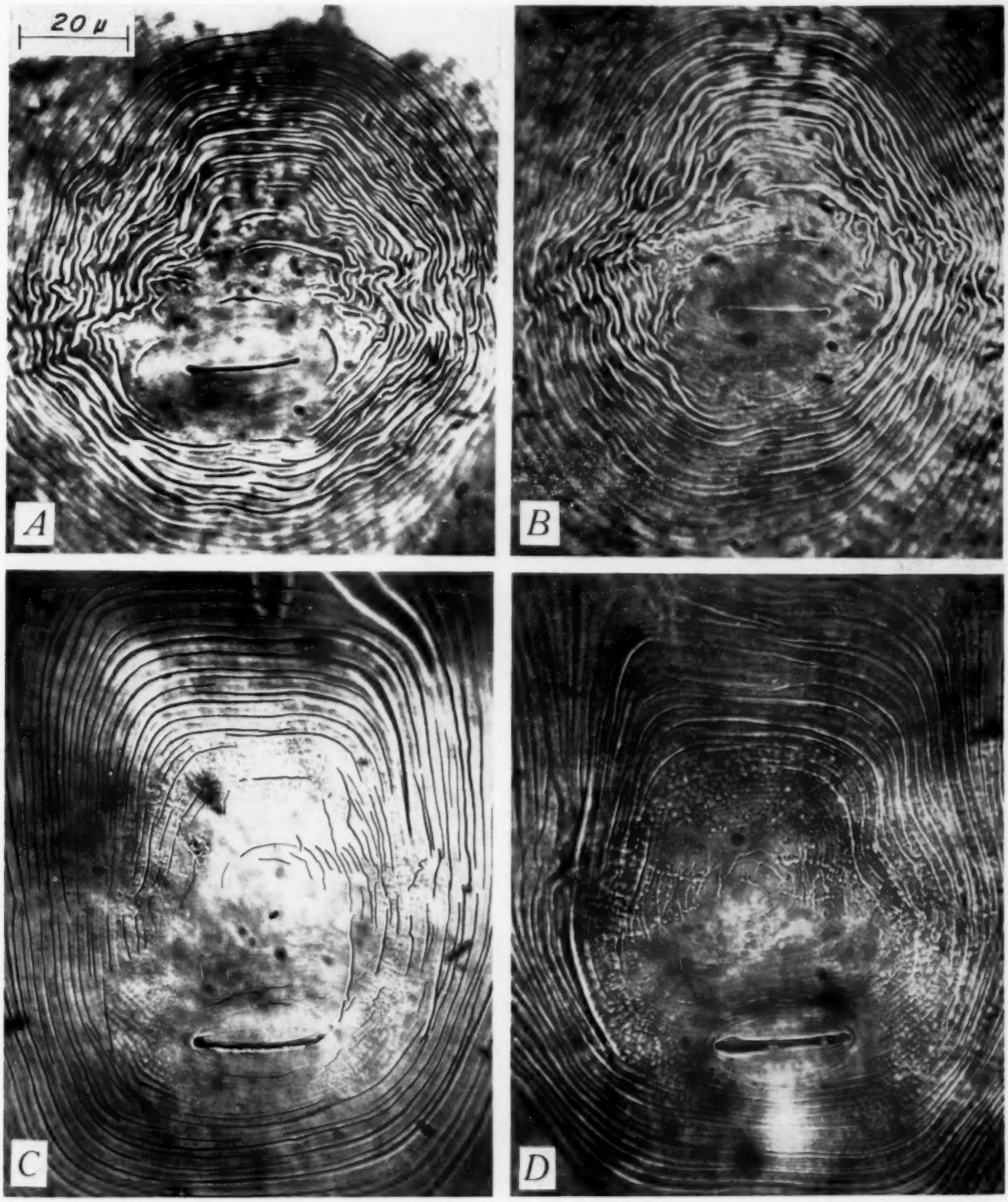

FIG. 8. A and B. Perineal patterns of *Meloidogyne exigua* from coffee root, Peru. C and D. Perineal patterns of *Meloidogyne brevicauda* from tea root, Ceylon. (Collected by C. A. Loos).

tion is examined.

The typical perineal pattern and the most common variations of each known form are described below. In each case, the first of the series of photographs of patterns for each form was selected as typical and the remainder as the most common variations.

Meloidogyne javanica (Treub, 1885) Chitwood, 1949. (Figs. 1 and 2, A-D).—The lateral lines of this

species are distinct incisures cutting through the striae of the perineal pattern so that few or no striae extend unbroken from the dorsal to the ventral sector. Often the lateral lines can be traced by irregularities in the striae for some distance to both sides of the pattern (Fig. 2, C). The striae in the ventral sector are smooth. Often there is a more or less distinct whorl at the tail tip (Fig. 2, B). In the typical pattern, the arch is low, but it may be high in variants (Fig. 2, D).

Meloidogyne hapla Chitwood, 1949. (Fig. 3, A-D).—The lateral lines may be marked only by slight irregularities in the striae (Fig. 3, A), or the striae of the dorsal and ventral sectors may meet at an angle along the lateral lines (Fig. 3, B-C). The arch is low. Near the tail tip there is often an area with distinct stippling or punctation (Fig. 3, A-C) which is not found in other species. The ventral striae are sometimes extended laterally to form wings on one or both sides (Fig. 3, B). The striae in both sectors are smooth or slightly wavy.

Meloidogyne arenaria (Neal, 1889) Chitwood, 1949. (Fig. 4, A-D).—The lateral lines are usually marked only by slight irregularities in the striae, but sometimes the dorsal and ventral striae may meet at an angle along the lateral lines. Near the lateral lines, there are numerous short disordered striae (Fig. 4, A). The arch is low and the pattern has a more or less circular outline except when the striae are extended laterally to form wings (Fig. 4, C). The perineal pattern of this species resembles that of *M. hapla*, but never has the punctate or stippled area near the tail tip.

Meloidogyne arenaria thamesi Chitwood, 1952. (Figs. 5, A-D).—In the typical pattern, the arch is low and flattened dorsally. One or both of the lateral lines may be distinct and bordered by short, irregular striae near the tail tip (Fig. 5, D). The ventral striae are sometimes extended laterally to form wings (Fig. 5, B and D).

Meloidogyne incognita (Kofoid and White, 1919) Chitwood, 1949. (Fig. 6, A-D).—The perineal pattern is roughly oval with a high, ill-formed arch composed of closely spaced, wavy to zig-zag striae. Along the lateral lines, there is a strong tendency toward forking of both dorsal and ventral striae.

Meloidogyne incognita acrita Chitwood, 1949. (Fig. 7, A-D).—In the typical pattern, the arch is high and composed of widely spaced wavy striae. Along the lateral lines there is some tendency toward forking of the striae and often some short striae (Fig. 7, A). The pattern, particularly near its edge, is made up of fine, evenly spaced striae interspersed with irregularly spaced, short, coarse striae (Fig. 7, B). Variants of the pattern of this subspecies are very similar to patterns of *M. incognita*.

Meloidogyne exigua Göldi, 1887. (Fig. 8, A-B).—The arch is low and slightly flattened. The lateral lines are poorly defined and bordered by folded and broken striae.

Meloidogyne brevicauda Loos, 1953. (Fig. 8, C-D).—The perineal pattern is roughly rectangular in outline with a high, flattened arch composed of widely spaced striae. The lateral lines are marked only by a few breaks in the striae. In the ventral sector, the striae are more closely spaced than in the dorsal sector.

KEY TO THE TYPICAL PERINEAL PATTERNS OF MELOIDOGYNE SPECIES

1. Arch low and rounded or flattened dorsally (Figs. 2, 3, 4, 5 and 8, A-B) 2
   Arch high, pattern roughly oval (Figs. 6, 7) or roughly rectangular (Figs. 8, C-D 6
2. Lateral lines distinct incisures often extending beyond edge of pattern. Few or no striae extending unbroken from dorsal to ventral sector (Fig. 2) ..... *Meloidogyne javanica*
   Lateral lines not distinct incisures, but marked only by irregularities or discontinuities in striae (Figs. 3, A, 4, 5, A-C and 8, A-B); or with striae meeting at an angle along lateral lines (Figs. 3, B-C, and 5, D) 3
3. Arch more or less rounded (Figs. 3 and 4) 4
   Arch flattened (Figs. 5 and 8, A-B) 5
4. Often with stippled or punctate area near tail tip. Without irregular striae near lateral lines (Fig. 3) ..... *Meloidogyne hapla*
   With irregular striae near lateral lines. Never with stippled or punctate area near tail tip (Fig. 4) ..... *Meloidogyne arenaria*
5. Striae folded near lateral lines (Fig. 8, A-B) ..... *Meloidogyne exigua*
   Lateral lines bordered by numerous short striae near tail tip (Fig. 5) ..... *Meloidogyne arenaria thamesi*
6. Pattern roughly oval (Figs. 6 and 7) 7
   Pattern roughly rectangular (Fig. 8, C-D) ..... *Meloidogyne brevicauda*
7. Dorsal striae closely spaced, wavy to zig-zag, arch often ill-formed (Fig. 6) *Meloidogyne incognita*
   Dorsal striae smooth to wavy, arch usually well-formed (Fig. 7) ..... *Meloidogyne incognita acrita*

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RESISTANCE OF CABBAGE TO BLACKROT[1]

Douglas C. Bain[2]

SUMMARY

Healthy plants were selected from among seedlings developing from inoculated seed of the varieties Huguenot, Early Fuji, and Round Dutch. Results of greenhouse and field inoculation tests of inbreds demonstrated resistance to blackrot. Resistance of the Round Dutch selection was broken down in severe inoculation tests but that of the other 2 selections was not. Progeny of crosses between Huguenot and diseased commercial varieties did not develop symptoms of blackrot in severe inoculation tests. Results of seed inoculation tests of F_2 progeny of some of these crosses suggested simple Mendelian inheritance with blackrot resistance controlled by a single dominant factor.

The possibility that selection for resistance to blackrot of cabbage caused by *Xanthomonas campestris* (E. F. Sm.) Dows. could lead to development of resistant varieties has been pointed out.[3] That such resistance might be based upon a genetic factor (or factors) was indicated later.[4,5] This paper describes in detail evidence obtained thus far.

EXPERIMENTAL RESULTS.—Healthy plants were selected from among diseased seedlings of the varieties Huguenot, Round Dutch and Early Fuji grown from seed which had been soaked in inoculum, incubated in Petri dishes, and planted in pots of fumigated soil in the manner previously described.[3] Inbreeding and selecting in each variety was continued through 2, 2, and 1 generations, respectively, before it was field-tested or crossed with susceptible plants.

The first field test was started in August 1952. Plants from each selection and from commercial seed of Round Dutch were set in the field in randomized replicated plots. Because of dry weather and high temperatures, the plants were irrigated by sprinkling every 10-14 days. During 3 weeks, all plants were inoculated with the blackrot organism a total of 5 times —3 by spraying and 2 by injection into the stem just below the terminal bud. A 4-day culture of bacteria grown in solution was used as inoculum. Symptoms of blackrot on leaf margins of the control variety were evident 12 days after the first inoculation, and progressed until the plants were severely fired or killed. Conversely, plants of the selections of Huguenot and Early Fuji were remarkably free of blackrot throughout the season. The selection of Round Dutch was slightly susceptible; development of disease symptoms was slow and there was considerably less firing of leaves than in the control variety. Disease grades, based upon a modification of Walker and Hare's[6] method, were recorded about 4 weeks after the last inoculation. The disease index of commercial Round Dutch was 64.2 and that of the Huguenot selection was 0.2 (Table 1).

Another field test of the resistant selections was made in the spring of 1953. Copenhagen Market from commercial seed was used as the susceptible control, and the Round Dutch selection was omitted because of an insufficient amount of seed. These plants were inoculated 3 times (erroneously reported[5] as 5). Inoculum was injected into the potted plants in the greenhouse a week prior to setting in the field in January to insure optimum temperature for development of the pathogen. Inoculum was sprayed on twice within 8 weeks after transplanting. Conditions were ideal for development and spread of bacteria throughout the growing season, as evidenced by the early and severe disease development of the susceptible control. Foliage disease notes were taken 13 days after the last inoculation. The disease index of Copenhagen Market was 82.0 (Table 1) but that of the Huguenot and Early Fuji selections was 0.2. One plant, out of 49 of each of the latter 2 selections, was given a score of 10 because of a trace of firing on the margin of one leaf. This, however, could have been due to something other than blackrot. Resistant reaction of young plants is illustrated in Figure 1.

TABLE 1.—*Calculated disease index of cabbage varieties and lines in blackrot field tests*

Line or Variety	Fall 1952	Spring 1953
Round Dutch	62.40[a]	----
Huguenot selection	0.20	0.20
Early Fuji selection	1.62	0.20
Round Dutch selection	14.70	----
Copenhagen Market	----	82.0
LSD @ 1% level	15.18	7.63

[a] 0—No external disease symptoms
10—Trace
40—Light
70—Moderate
100—Severe to killing

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[2] Present address: Department of Plant Pathology and Physiology, State College, Mississippi.

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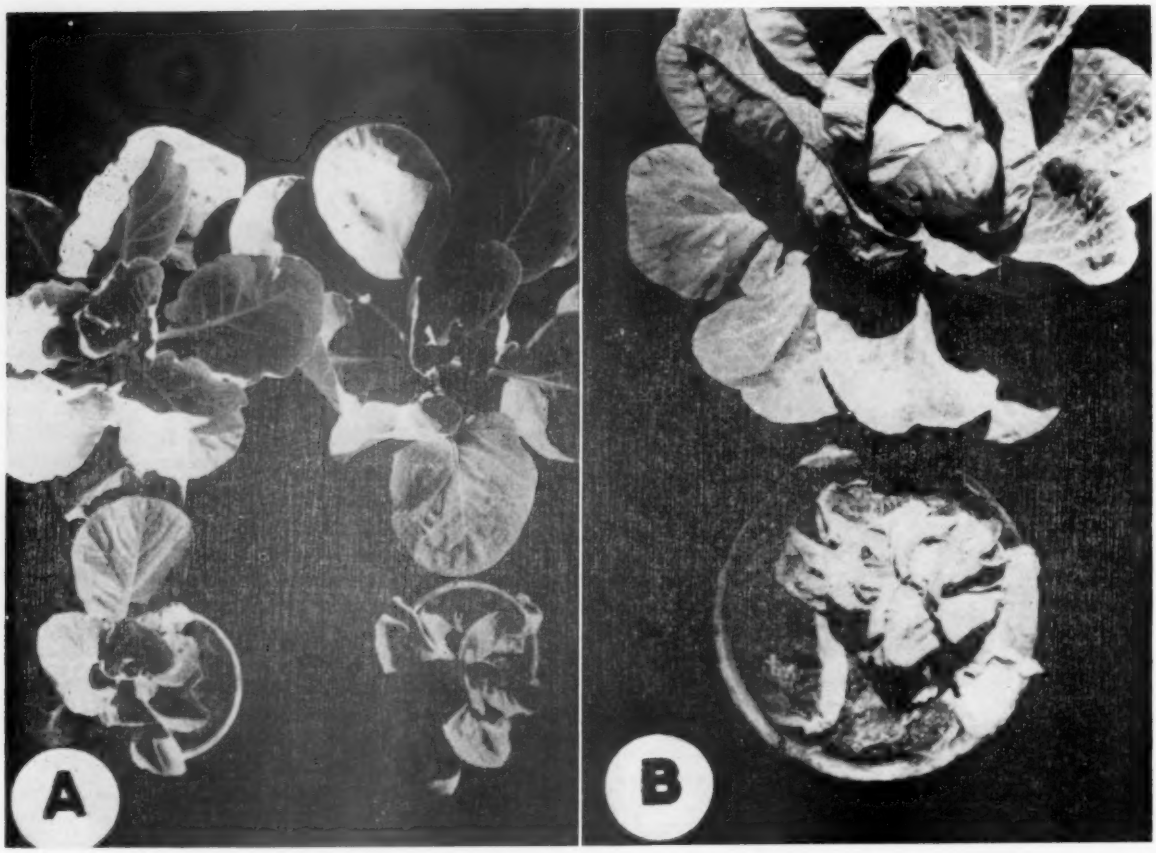

FIG. 1. Blackrot resistant and susceptible cabbage 4 weeks after inoculum was injected (A) and 10 weeks after (B). Plants about 5 weeks old when inoculated. Top: Huguenot; bottom: Copenhagen Market.

Shortly after foliage disease readings were made, stems of the plants were cut diagonally, as nearly through the injection scar as possible, to determine extent and severity of vascular discoloration. The vascular systems of all plants of the control variety were uniformly blackened. In the selections, however, the percentage of discolored stems was considerably reduced (Huguenot 16.6 per cent, Early Fuji 4.0 per cent), and in these there were only a few scattered discolored strands—apparently confined to the sides of the stems where the needle entered or came out. No tissue platings were made to determine presence of the pathogen.

Plants of the F_1 generation from susceptible × resistant crosses were tested for reaction to blackrot. Progeny of the second inbred generation of Huguenot were crossed with Early Jersey Wakefield and Bonanza from commercial seed. Diseased plants were used as female parents, and were not pure-lined for susceptibility. Progeny of these crosses were inoculated twice by injection in 24 hours—considered to be a rather severe test. The plants were about 5 weeks old and were in pots in a greenhouse. Progeny of selfed susceptible parents and of a resistant selection were used as controls. Temperature and moisture conditions were favorable for development of blackrot. All plants (Table 2) of the susceptible control varieties developed clear and distinct symptoms of systemic blackrot within 8 days after the first injection, whereas those of the resistant control and F_1 plants of the crosses had failed to develop disease symptoms 3 weeks after inoculation.

Seed inoculation tests were made with progeny of the F_2 generation of the crosses Early Jersey Wakefield × Huguenot and Bonanza × Huguenot. Seed of

TABLE 2.—*Reaction of F_1 (susceptible × resistant) plants to blackrot*

Variety	Plants with Blackrot	Plants Healthy
Early Jersey Wakefield[a] × Huguenot selection	0	8
Bonanza[a] × Huguenot selection	0	17
Early Glory × Huguenot selection	0	12
Huguenot selection × Bonanza[a]	0	5
Early Jersey Wakefield	9	0
Bonanza	8	0
Huguenot selection	0	15

[a] Plant with blackrot.

TABLE 3.—*Results of seed inoculation tests of F_3 progeny of susceptible × resistant crosses and inbred resistant lines. Notes taken 3 weeks after planting*

Variety	No. Plants	Percentage Blackrot
Early Jersey Wakefield × Huguenot	134	18.6
Bonanza × Huguenot	160	35.6
Huguenot selection[b]	200+[a]	0.0
Early Fuji selection[c]	205+	2.4
Round Dutch selection[b]	224+	10.0

[a] Plus sign indicates more than 200 healthy plants remaining after diseased plants had been rogued.
[b] Progeny of 4th inbred generation.
[c] Progeny of 3rd inbred generation.

the third, fourth, and fourth inbred generations of Early Fuji, Round Dutch, and Huguenot, respectively, were included. No susceptible controls were used. The high degree of resistance (Table 3) of the Huguenot and Early Fuji selections was apparent, but Round Dutch selection again showed a degree of susceptibility. Results of the tests of progeny of the Early Jersey Wakefield cross suggested simple Mendelian inheritance with resistance controlled by a single dominant factor. It should be noted, however, that the susceptible parents of these crosses were not pure-lined for susceptibility but were diseased plants from commercial seed; also, despite the effectiveness of seed inoculation as a means of evaluating resistance,[7] this method is not so accurate as is desirable.

DISCUSSION.—Results of the 2 field tests, as well as seedling and injection tests, demonstrated blackrot resistance in cabbage. That this resistance is possibly of 2 types is suggested by the difference in disease grades of the Round Dutch selection and the 2 others in the 1952 field test and in seedling tests. There were only 6 plants out of 47 of the Round Dutch selection that were not rated 10 or more, and in seedling tests there was also an indication of slight susceptibility. On the other hand, 48 of 49 Huguenot and 41 of 49 Early Fuji plants were given a rating of 0 (the others were 10) and in seedling tests the percentage of diseased plants was very low. Thus, under severe testing conditions, resistance in the Round Dutch selection broke down somewhat but that of the other 2 selections did not. Similar results were observed in preliminary injection tests. Results of the 2 field tests, injection tests, and tests of progeny of crosses between Huguenot and susceptible varieties demonstrated that resistance in this line did not break down and suggested that it evidently is inherited as 1 or more dominant factors.

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[7] Bain, D. C. 1955. Disappearance of blackrot symptoms in cabbage seedlings. Phytopathology 45: 55-56.

ANTIBIOSIS AND THE RED ROT DISEASE OF SUGAR CANE [1]

B. A. Bourne

SUMMARY

A yeast identified as *Candida intermedia*, found associated internally and externally with the sugar cane moth borer and one of its parasites in the adult stage, has been found to be strongly antibiotic against the sugar cane red rot fungus *Physalospora tucumanensis*, but not against an associated red rot organism *Fusarium moniliforme*, commonly encountered in the Florida Everglades. The possibility of using *Candida intermedia* by cultivating it on sterile cane juice or dilute cane molasses for inoculating cane cuttings or for the production of toxin for the control of the red rot due to *Physalospora tucumanensis* is suggested.

Although sugar cane red rot disease is considered of major importance in most countries where sugar cane is grown and has been studied intensively by many pathologists, no record appears to have been made of any antibiotic microorganisms that affect the fungi associated with this malady and which conceivably could be utilized for its control.

It was during a recent study on the dissemination of sugar cane diseases (1) that an interesting case of antibiotic activity against the red rot fungus *Physalospora tucumanensis* Speg. came to light. A yeast identified as *Candida intermedia*, commonly associated with the sugar cane moth borer *Diatraea saccharalis* Fabr. and one of its parasites *Agathis stigmaterus* (Cross), has been found to be strongly antibiotic to *P. tucumanensis*. The constant occurrence of yeasts and other microorganisms in the gut of certain insects is well known, although their function is poorly understood. Wigglesworth (7) states that if these organisms are of value, it seems more likely that they contribute to nutrition or metabolism.

Because of the diverse views held by workers in different countries as to the importance of the cane borer in spreading red rot infection, it seemed obvious that some unknown factor or factors may be responsible for the differences of opinion. Tims and Edgerton (6) in Louisiana, considered that severe red rot infections were usually associated with attacks by the

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sugar cane moth borer *Diatraea saccharalis*. On the other hand Chona (3) in India stated that borers play little part in starting or spreading the disease.

The author has already noted (2) that 2 fungi, viz: *Colletotrichum falcatum* Went and *Fusarium moniliforme* (Sheld) Snyd. & Hans. are associated with sugar cane red rot in the Florida Everglades. These fungi frequently occur together in diseased tissues. By inoculating both fungi together in Petri plate cultures, it was observed on many occasions that they showed no antagonism to each other.

In view of the association of *Diatraea saccharalis* larvae with sugar cane red rot and the frequent presence of the yeast *Candida intermedia* with the moth borer internally and externally, it was decided to test its reaction to the growth of both red rot fungi.

Experimental methods.—In the first series of tests for antagonism, using 6 replications, a method similar to that described by Cooper and Chilton (4) was employed, planting the red rot fungus on one side of a 10 cm. diameter Petri plate containing 10 cc of "Difco" nutrient starch agar to which 10 per cent sugar cane stalk juice had been added by volume and streaking the yeast culture on the opposite side. Cultures were then incubated at 24°C. In the second series of otherwise similar tests, the red rot fungus culture was planted in the center of the plate and a ring of the yeast culture circumscribed around it about 3 cm. in diameter.

Results.—After incubation for 9 days, the plates inoculated with *Physalospora tucumanensis*, a virulent light strain, showed the red rot fungus inhibited along the lines of the streaks of *Candida intermedia*, but growing around the ends thereof. The plates inoculated with *Fusarium moniliforme* (purple strain) showed that the red rot fungus had grown past the streak yeast cultures without any inhibition being apparent. After further incubation for a total of 13 days, *P. tucumanensis* had not yet passed the yeast streak line in any of the plates whereas *F. moniliforme* had completely filled the Petri plates.

Fig. 1 shows typical results of the second test series after 8 days. Fig. 1.A shows the *P. tucumanensis* colony completely confined by the circle of *C. intermedia*. Even after 18 days it was still confined by the yeast circle. Fig. 1.B shows the *F. moniliforme* after 8 days, where it had grown past the circle of *C. intermedia* without any apparent inhibition. By the thirteenth day it had completely filled the Petri plate.

Discussion.—The fact that the yeast *Candida intermedia* is frequently found associated with the sugar cane moth borer both internally and externally, as well as with the adult stage of one of its parasites, indicates strongly that this organism in nature may be of considerable importance in curbing spread of *Physalospora tucumanensis* in borer holes, due to its strong antibiotic activity against it. In fact, it is entirely possible that this or a similar antibiotic organism might be active against *P. tucumanensis* in India and explain why moth borers are not involved in spreading the red rot caused by this fungus in that country. On the other hand, since the associated red rot fungus *Fusarium moniliforme* is not inhibited by this yeast, a partial explanation is afforded as to its prevalence in the Everglades of Florida, often in the absence of *P. tucumanensis*.

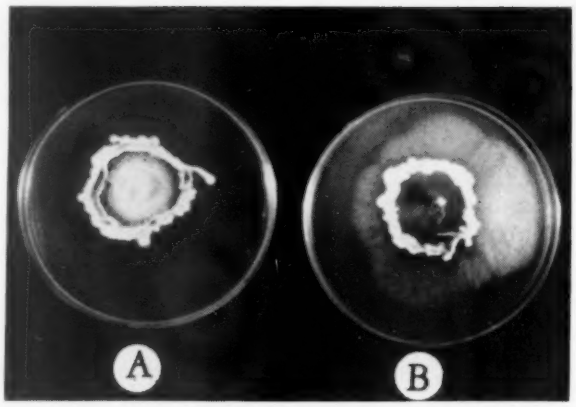

Fig. 1. Circles of *Candida intermedia* in plate cultures, the centers of which were inoculated with A) *Physalospora tucumanensis* and B) *Fusarium moniliforme*, after incubation at 24°C. for 8 days.

In view of the potency of the antibiotic toximycin against *Colletotrichum circinans* (Berk) Vogl as reported by Stessel et al (5), it would seem desirable to investigate its activity against *P. tucumanensis*, the imperfect stage of which is *Colletotrichum falcatum* Went. In this instance, the bacterial organism *Bacillus subtilis* is the source of the toxin.

Both *Candida intermedia* and *Bacillus subtilis* are easily cultivated in such liquid media as sterilized sugar cane juice or dilute sugar cane final molasses. Should either of these organisms be cultivated for direct stalk cutting or seedpiece inoculation, or for their toxic byproducts to be used in controlling the red rot fungus *Physalospora tucumanensis*, these cheap media are suggested.

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PHYSIOLOGICAL DIFFERENCES BETWEEN A NORMAL AND A DEGENERATE STRAIN OF SCLEROTINIA TRIFOLIORUM [1]

Victor M. Held

SUMMARY

The nutrition, protopectinase secretion, and toxin secretion of a normal and a degenerate strain of *Sclerotinia trifoliorum* Erikss. were studied to determine possible differences between the strains which might add to knowledge of the pathogen-suscept relation.

The effect of 27 sources of nitrogen and various B-vitamins, singly and in combination, on the growth of the strains was measured by the dry weight of the mycelial mats. Significant differences were found among nitrogen sources, and between strains. Biotin accelerated the initial growth of both strains; thiamine-HCl and inositol did not.

The degenerate strain secreted more protopectinase than the normal strain in various synthetic media. Sodium polypectate increased the secretion of this enzyme by both strains.

The normal strain secreted a substance that caused wilting of clover leaves under conditions inducing rapid transpiration, but the degenerate strain did not.

Sclero inia trifoliorum Erikss. causes a clover rot, which results in considerable loss wherever the crop is grown. If the fungus is allowed to grow on potato dextrose agar for several weeks without being transferred, it may undergo an apparently irreversible change after which sub-cultures started from the mycelium yield a variant that cannot attack clover. The variant does not form sclerotia; consequently, no apothecia or ascospores are produced. Degenerate variants of *S. trifoliorum* have been observed by Frandsen (3) and Bjorling (2). Using a normal strain of the fungus, and a degenerate variant obtained from it, a series of physiological tests were conducted to reveal any differences between them that might help explain why one is virulent, and the other not. By the study of these closely related strains, only one of which (the normal) can attack clover, a better understanding of the pathogen-suscept relation was sought.

MATERIALS AND METHODS.—The organism used was *Sclerotinia trifoliorum* Erikss., strain R-1-3, and its degenerate variant, hereafter referred to as the normal and the degenerate strain, respectively. The normal strain was obtained from a single ascospore. Both were supplied through the courtesy of Dr. K. W. Kreitlow,[2] U.S.D.A., Beltsville, Maryland. Inoculation of red clover seedlings proved that the normal strain was virulent and the degenerate strain was not.

Stock cultures were grown on Difco potato dextrose agar and maintained by transfer every 5 days. Inoculum was produced in the same manner for all but the tests in which the effects of certain B-vitamins were considered. In the latter case, inoculum was grown on a medium composed of 0.5 g $MgSO_4 \cdot 7H_2O$, 2.0 g KH_2PO_4, 3.0 g NH_4NO_3, 0.01 g $FeSO_4$, 20 g D-glucose, 1000 ml distilled water, and 20 g Difco agar purified according to the procedure outlined by Lilly and Barnett (4).

The incubation temperature for stock cultures, and for growth of the organism in all the tests was 15° ± 1°C. Each culture vessel was inoculated with a single 3 mm disc of mycelium from the edge of a spreading culture. Liquid media were employed in all the nutritional studies, and growth was measured on the basis of the dry weight of the mycelium. Media were adjusted to pH 5.5 with 0.5N KOH or HCl before autoclaving.

The basal medium used in the study of nitrogen sources was composed of 0.50 g $MgSO_4 \cdot 7H_2O$, 2.00 g KH_2PO_4, 3.75 g anhydrous D-glucose, 0.01 g $FeSO_4$, and 1000 ml distilled water. Each nitrogen source was added in the amount needed to supply 250 mg nitrogen per liter of medium. Where pairs of amino acids were used, a total of 500 mg of nitrogen per liter was supplied. The culture vessels were 125 ml Erlenmeyer flasks each containing 25 ml of medium. Sugar determinations on used media were done according to Sullivan's (9) modification of Phillips' method, after clarification with lead acetate, the excess of which was removed with sodium sulfate.

To observe the effects of vitamins on growth of the fungus, vitamin-free casein hydrolysate was added to the basal medium to supply nitrogen in the amount indicated above. Thiamine-HCl, biotin, and inositol were added singly, and in all possible combinations, at the rate of 0.1 mg, 0.025 mg, and 1.0 mg per liter, respectively. The vitamin-free medium was used as a check. Mats were harvested after 12 days' incubation.

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[2] Unpublished observations by K. W. Kreitlow and mentioned in the 1948 and subsequent Annual Reports of the U. S. Regional Pasture Research Laboratory.

The protopectinase activity of various solutions was tested by noting the time required for maceration of discs of potato tuber 16 mm in diameter, and 0.5 mm thick at 25°C. The mean of 3 replicates was used. The action on potato discs of pectinase obtained from Nutritional Biochemicals Corporation and Taka-diastase purchased from Parke Davis and Co. was determined. The commercial pectinase is a mixture of pectic enzymes of fungus origin. Taka-diastase is a mixture of starch-splitting enzymes. Solutions of these enzyme concentrates were made by dissolving 1 g of the powder in 100 ml of distilled water, and tested over a pH range of 3.0–8.0, inclusive. The effect of low pH alone was tested by placing the discs in solutions of HCl. In view of the findings, all the enzyme solutions were tested at pH 3.0 or above, and the tests were not allowed to run more than 12 hours. Protopectinase activity was considered negligible when loss of coherence failed to occur within that time.

The solutions tested were 1) the juice squeezed from autoclaved potato cylinders on which the fungus had grown, 2) solutions of precipitates obtained by adding 3 volumes of ethyl alcohol to potato dextrose broth in which the fungus had grown, and 3) used media consisting of inorganic salts and either D-glucose or sodium polypectate. The alcohol precipitates were dried over $CaCl_2$, ground in a mortar, and tested at the rate of 1 g per 100 ml of water.

D-glucose or sodium polypectate (product #24A4 of the California Fruit Growers Exchange) was substituted in the basal medium (with 2 g NH_4NO_3 per liter as the nitrogen source) at concentrations of 5, 10, and 30 g per liter. Used media from 1 flask in each group were tested at 2-day intervals from the tenth to the twentieth day of incubation. Each sampling was accompanied by a test with commercial pectinase as a check against variation in the tubers. The used media were adjusted to pH 3.0 before testing.

To determine whether a toxin was secreted, after 20 days' incubation in the basal medium (with NH_4NO_3), the mycelial mats were removed, washed in sterile distilled water, and placed in fresh media where they were incubated for 48–96 hours. These are referred to as replacement cultures. Both the original filtrates, and those from replacement cultures were tested for the presence of a toxin by noting their effect on Ladino clover leaves. Fully turgid leaves with petioles about 6 in. long were placed in the filtrates and checked for wilting.

EXPERIMENTAL RESULTS. — *Nitrogen sources.* — The growth of the strains with NH_4NO_3, D-arginine-HCl, DL-alanine, DL-isoleucine, L-aspartic acid, DL-lysine, and DL-threonine was measured at 6-day intervals over a period of 6–30 days. The twelfth day of incubation corresponded to a period of rapid growth with all but DL-lysine which gave very poor growth, and DL-threonine which gave rapid growth between 18–24 days with the normal, and 24–30 days with the degenerate strain. Growth differences with the relatively good nitrogen sources were greatest after 12 days' incubation.

With regard to the pathogen-suscept relation, a measurement of initial growth seemed important, for it would indicate to what extent a nitrogenous compound is immediately utilizable. Table 1 gives the nitrogen sources used, and the mean dry weights of the fungus mats for each strain. The least significant differences at the 5 per cent and 1 per cent levels, respectively, are as follows: for the normal strain, 3.9 mg and 5.2 mg; for the degenerate strain, 4.3 mg and 5.7 mg; for strains × nitrogen source interaction, 8.2 mg and 10.9 mg. The differences in growth in favor of the normal strain were highly significant with L-asparagine, L-proline, and NH_4Cl; significant with vitamin-free casein hydrolysate.

By supplying glycine, DL-threonine, and DL-lysine which gave poor growth, with DL-alanine or D-arginine-HCl which gave good growth, the normal strain grew more, and the degenerate strain grew as much as when the last 2 amino acids were supplied singly after 24 days' incubation. This indicates that the first 3 amino acids are not readily utilizable rather than toxic at the concentrations used.

In potato dextrose broth, (nitrogen content 413 mg per liter as determined by the Kjeldahl method) the normal strain produced about 30 per cent more mycelium than the degenerate after 12 days' incubation whether 10, 15, or 30 g per liter of D-glucose were added. The initial growth of both strains was much greater than that obtained in any synthetic media used.

TABLE 1.—*Average dry weight in mg of mycelial mats of S. trifoliorum produced after 12 days' incubation at 15°C in 25 ml of basal medium with various sources of nitrogen*

Nitrogen source	Dry weight (mg) of strains	
	Normal	Degenerate
NH_4Cl	22	11
NH_4NO_3	18	14
$NaNO_3$	1	1
$Ca(NO_3)_2 \cdot 4H_2O$	4	3
Glycine	5	5
DL-alanine	16	17
DL-valine	6	9
DL-threonine	3	4
L-leucine	21	22
DL-isoleucine	11	11
DL-norleucine	15	9
DL-serine	7	8
L-cystine	7	3
DL-methionine	8	8
L-aspartic acid	12	16
L-asparagine	31	15
D-glutamic acid	17	21
Phenylalanine	26	28
L-tryptophan	5	5
L-proline	26	15
L-histidine-HCl	14	17
D-arginine-HCl	21	19
DL-lysine-HCl	2	2
3,5 diiodotyrosine	0	0
Acetamide	7	5
Casein hydrolysate	38	29
Urea	2	2
None	0	0

Growth differences between the strains cannot be attributed to pH changes. Both are quite acid tolerant. In all media used, the pH dropped to 3.5–3.0 by the twelfth day of incubation. The pH drifts induced by the strains in any given medium were almost identical.

Tests for reducing sugar on used basal medium plus various nitrogen sources revealed a difference in behavior of the strains. After 30 days' incubation, filtrates of the normal strain contained appreciable quantities of reducing sugar when NH_4NO_3, D-arginine-HCl, and DL-alanine supplied nitrogen. The filtrates of both strains gave negative tests for reducing sugar when nitrogen was supplied by DL-isoleucine, or L-aspartic acid.

Growth of the strains could not be reliably compared on the basis of mat diameter on solid media. The mean diameter of the mats produced by the normal strain after 6 days' growth on potato dextrose agar was 45 per cent greater than those produced by the degenerate strain, yet there was no significant difference in the dry weight of mycelium produced by the 2 strains.

Response to B-vitamins.—Both strains can grow without vitamins in the substrate. Growth of both strains was significantly greater in the presence of biotin than with no vitamins. The growth of the normal strain was significantly greater than that of the degenerate with all except the thiamine-HCl + biotin combination. The greatest difference between strains occurred with biotin + inositol.

Secretion of protopectinase.—There was no maceration of potato discs in extracts from autoclaved potato cylinders on which either of the strains had grown, or in solutions of precipitates obtained by adding ethyl alcohol to potato dextrose broth in which the strains had grown.

In synthetic medium containing D-glucose or sodium polypectate as the carbon source, the degenerate strain secreted considerably more protopectinase than the normal strain. Sodium polypectate increased the secretion of this enzymes by both strains. Protopectinase secretion by the degenerate strain increased markedly with increase in sodium polypectate concentration but seemed unaffected by the concentration of D-glucose. The normal strain secreted more protopectinase at the lower concentrations of both carbon sources.

Toxin secretion.—Both the original filtrates and replacement culture filtrates from the normal strain contained a substance that caused wilting (within 45 minutes) of Ladino clover leaves under conditions inducing rapid transpiration. Wilting was rapid in bright sunlight, at 20°–26°C. Wilting did not occur on a cloudy day at 18°C. Wilted leaves transferred to water after 5 hours recovered except for occasional desiccated areas on the leaflets. The toxic principle was not destroyed by heating to 100°C for 10 minutes. There was no wilting of the leaves in filtrates from the degenerate strain, nor in unused medium.

An occasional leaf that did not wilt suggested a possible quick method of testing for resistance in clones. However, leaves from 2 clones that showed resistance and susceptibility in field and greenhouse tests wilted in like proportion.

Discussion.—To be pathogenic, an organism may utilize enough food to starve the suscept cells, it may secrete metabolites that induce abnormal physiological activity in the cells, or enzymes that evoke chemical reactions resulting in physical breakdown. Whether the result is death or one of the hyperplastic or hypoplastic symptoms depends on the kind and degree of physiological disturbance. Nutritional studies *per se* may be more useful in explaining parasitism, which is a food relationship, than pathogenicity, which is a disease relationship. Several instances of parasitism unaccompanied by pathogenicity have been observed (1). Therefore, the enzyme and toxin approach have also been considered in this work.

Growth of the strains with various sources of nitrogen suggests that *S. trifoliorum* belongs in Robbins' (8) group 3, i.e., fungi which grow only on NH_4^+, and organic nitrogen. This agrees with the findings of Pape (6) who tested the effects of 8 nitrogenous compounds on several normal strains of the fungus. Other results are difficult to compare with those of Pape, for he used relatively concentrated media solidified with agar, which introduces certain growth factors. Also, he rates growth subjectively.

The growth obtained with pairs of amino acids, casein hydrolysate, and potato dextrose broth, which probably contains nitrogen as protein, shows that the growth of both strains, but especially the normal, is stimulated by complex nitrogenous compounds.

Sugar determinations on used basal medium containing NH_4NO_3, D-arginine-HCl, or DL-alanine as the source of nitrogen reveal that under these conditions 1) the normal strain uses less of the carbon source than the degenerate in producing a unit weight of mycelium, or 2) the reducing substance is not the D-glucose originally supplied, but a carbohydrate secreted by the normal strain. It may be the substance that causes wilting in clover leaves.

Since only 2 strains of *S. trifoliorum* were used in these tests, it is possible that the nutritional differences found do not exceed those which might be found among normal isolates. Pape found marked variations in growth among normal isolates, but, as noted above, it is difficult to compare his results with those presented here.

Both strains secreted protopectinase, but the degenerate strain produced more than the virulent. Possibly the activity of the normal strain in this regard is different when the fungus is acting on its suscept, as shown by Menon (5) for *Pythium* and *Phytophthora* species. The fungi secreted pectic enzymes only when grown on plant tissue.

The most significant difference between the strains is the secretion of a substance by the normal strain that causes wilting of clover leaves under conditions inducing rapid transpiration. Wilting is the first symptom of *Sclerotinia* attack as seen in the field. This suggests

a rapid method of testing for resistance in clones if resistance is due to some property residing in the protoplasm, and not to mechanical factors such as toughness of the epidermis. The fact that leaves of a resistant and a susceptible clone used in this work wilted in like proportion does not rule out the possibilities of such a test. The leaves of the susceptible clone were large and tender; the resistant clone was considerably smaller and tougher in texture. Resistance may have been due to anatomical factors. The basis for resistance to *S. trifoliorum* is not understood. Valleau et al (10) postulated that it might be due to a more efficient use of food reserves by certain clover varieties, resulting in the formation of a middle lamella that is less easily hydrolyzed by the enzymic activity of the fungus. Pohjakallio (7) suggests that chlorophyll affects resistance either directly, or indirectly through its connection with the assimilation mechanism.

Four reasons may be postulated to account for the virulence of the normal strain: 1) it grows more vigorously than the degenerate strain, particularly in the presence of complex organic compounds, 2) it secretes a metabolite that causes wilting of clover leaves, 3) it secretes protopectinase, though in smaller quantities than the degenerate strain, and 4) it may secrete other enzymes, not tested for here, which enable it to penetrate the tissues of its suscept.

It should not be overlooked that the question: "What makes a fungus pathogenic?" has its counterpart in the question: "What makes a plant susceptible?" Considering the thousands of known pathogens, immunity is the general condition in nature and therefore a physiological study of the suscept is also needed. The greatest obstacles to such an investigation reside in the lack of techniques for studying the disease process *in vivo*, and in the proper evaluation of *in vitro* studies.

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ACCUMULATION OF CHEMICALS IN DISEASED AREAS OF LEAVES [1]

C. E. Yarwood and Louis Jacobson [2]

SUMMARY

Leaves infected with a variety of pathogens (viruses, downy mildews, powdery mildews, rusts, leaf spot fungi) were exposed to vapors from Na_2S^{35}, to $H_3P^{32}O_4$, and C^{14} sucrose in a variety of ways and the accumulation of radioactivity was followed by means of autoradiographs and in a Geiger-Muller counter. In direct application of the chemicals to the leaves there was a greater accumulation of the radiochemical in the diseased than in healthy tissue in 19 host-pathogen combinations, lesser accumulation in 6 cases, and no clear difference in 6 cases. With local lesions of tobacco mosaic virus on *Nicotiana glutinosa*, 19 times as much S^{35} accumulated in 5-day lesions as in healthy tissue. With rust lesions on bean the S^{35} accumulated in a zonate pattern.

In experiments where C^{14} and P^{32} were accumulated via translocation, the degree of selective accumulation in rusted bean leaves was much greater than in the cases of direct application of the radiochemicals to the diseased leaves. In one case with a healthy bean leaf in $H_3P^{32}O_4$ and the opposite half-rusted leaf in water, 7870 times as much P^{32} accumulated in the rusted as in the healthy portion of the same leaf. Selective accumulation occurred, but to a lesser extent when the rust pathogen was killed in the living leaf.

It is suggested that the high metabolic activity of the pathogen or infected host cells might account for the observed selective accumulation.

The ability of diseased plant tissues to accumulate certain chemicals to a greater extent than similar healthy tissues has been observed since staining techniques were first applied to diseased tissues. Use of radioisotopes has made it possible to study the problem qualitatively and quantitatively with a variety of compounds. Some preliminary results have been reported (9, 10).

Selective accumulation by diseased leaf tissue may be defined as the greater accumulation of externally applied chemicals in diseased than in comparable normal tissues. A measure of the selective accumulation is the accumulation ratio, the ratio of the amounts of chemical accumulated in similar areas of diseased and healthy tissues.

METHODS.—The radioelements used to measure selective accumulation were S^{35}, P^{32} and C^{14} in the form of H_2S, H_3PO_4, and sucrose. It is believed that in all cases the radioactivity of the applied dosage or the period of exposure was sufficiently low to cause no important physiological changes in the tissue.

The S^{35} was obtained as BaS^{35} in an aqueous solution of $Ba(OH)_2$. This was diluted with 1 per cent $Na_2S \cdot 9H_2O$ + 0.1 per cent KOH. Labeled H_2S was generated in sealed pint jars by adding borate-phosphate buffer, pH8, to portions of the stock solution. The total volume was 50 ml. The amount of S^{35} in these test solutions varied from 5 to 250μ c and the amount of inert Na_2S varied from 0.001 to 0.04 per cent Na_2S. Usually one leaf, weighing 0.5–1 g fresh weight, was supported on a glass cylinder over the test solution. After exposure to the labeled H_2S, autoradiographs were prepared by placing the leaves between no-screen X-ray films at 0°C for periods ranging from 1 to several days. For quantitative assay of activity, the leaves were pressed flat and dried at 70°C. The activity was determined by placing the dried leaf under a lead disc with a hole approximately the area of the disease lesion. The lead discs were of sufficient thickness (1.4g/cm^2) to block completely all radiation except that transmitted through the hole. Counts of activity in a GM counter were made on similar areas of healthy and diseased tissues in the same leaf. Usually single lesions were chosen, their activity measured, and then similar areas of healthy tissue well removed from the lesions were measured. Although self absorption was a factor in measuring the soft radiation from S^{35} and C^{14}, it is believed that the thickness of the compared areas (approximately 3 mg/cm^2) was sufficiently low and uniform to permit valid comparisons.

P^{32} was obtained as very high specific activity $H_3P^{32}O_4$ and was diluted to 0.2–1μc/ml for use. No carrier was added. The C^{14}-sucrose was used at concentrations varying from 1–10 per cent sucrose and containing 0.05μc/ml. Leaves were exposed to the $H_3P^{32}O_4$ or C^{14}-sucrose solutions by floating infected detached leaves on the test solution, or by completely immersing leaves in the test solution in translocation experiments. After treatment, the leaves were washed and dried, and the healthy and diseased areas assayed for radioactivity.

The host-pathogen combinations studied will be given later. All infections on bean, tobacco, and cucumber were from artificial inoculations in the greenhouse. All others were from field collections. Most of this study was with bean rust on primary bean leaves at 5–8 days after inoculation, and this is the disease referred to unless otherwise indicated. The bean rust mycelium is entirely intercellular and causes no apparent necrosis. Bean powdery mildew was also used, and with this as with other powdery mildews studied, the mycelium is entirely superficial, with only

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[2] The writers acknowledge the assistance of A. H. Gold in preparing the C^{14} sucrose and 2 autoradiographs.

haustoria extending into the epidermal cells of the host.

In studies of the selective accumulation after translocation, a variety of methods was used as follows: a) the solution containing the radioactive chemical was applied to the soil, b) cut stems of detached plants were placed in the radioactive solution, or c) 1 of the 2 opposite primary bean leaves was immersed in the radioactive solution. In the last type of exposure the plants were with or without roots. They were kept in the natural light of the greenhouse or in total darkness. The leaf opposite the treated leaf was exposed to air or immersed in water (Fig. 11, 12). Leaves of treated plants were entirely healthy or entirely rusted or half healthy and half rusted in a variety of combinations. Half rusted leaves are those of which the lamina on only one side of the midrib was inoculated.

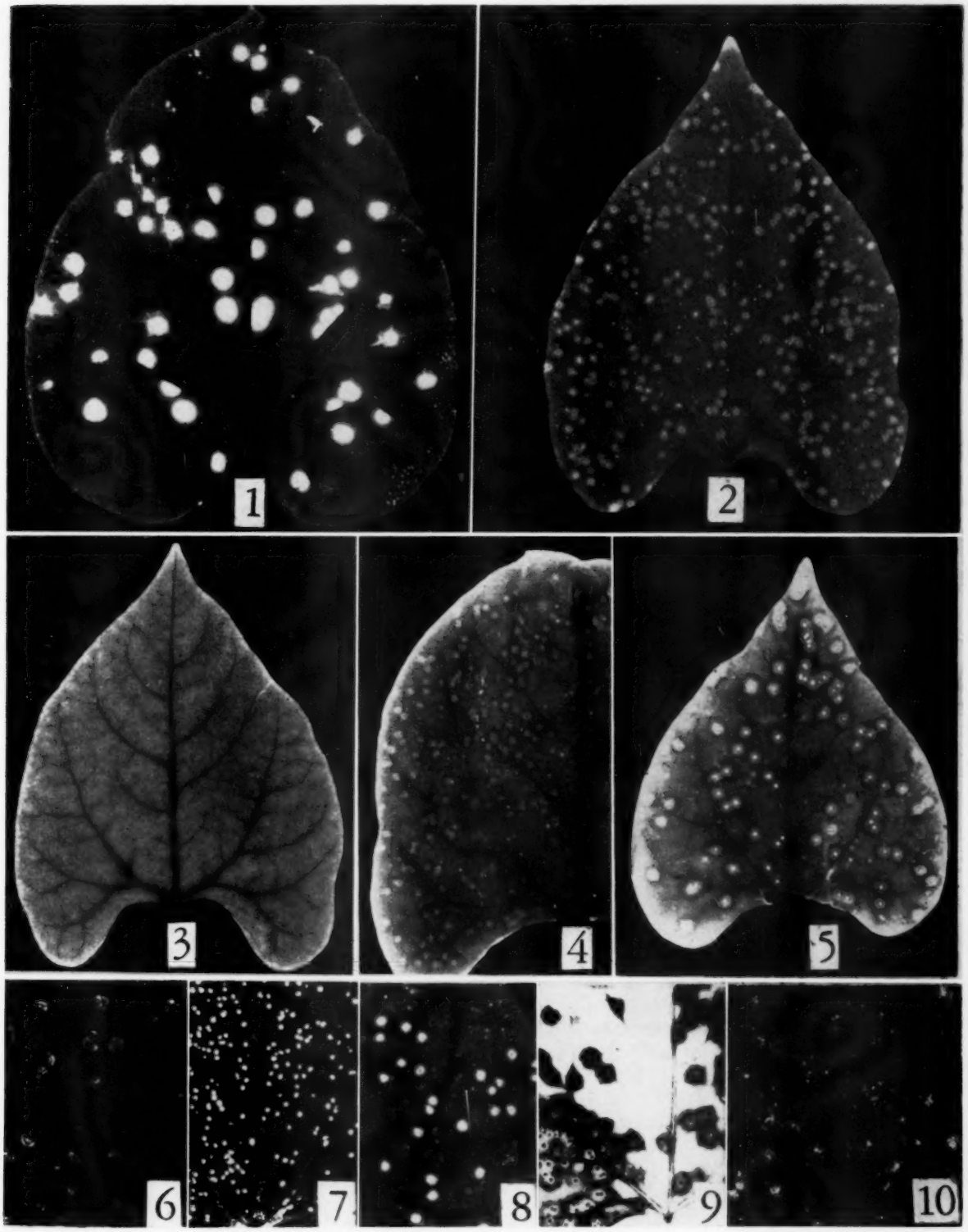
In order to obtain reliable counting data for the amounts of C^{14} and P^{32} translocated and accumulated, the radioactivity of several square centimeters of leaf was measured. To secure extensive but closely comparable areas of healthy and rusted tissue, bean leaves were inoculated heavily to give about 100 pustules/cm^2 on one side of the midrib while the opposite half of the leaf was not infected. With pustules so close together, the problem of low peripheral accumulation around the pustules as observed with localized lesions (9) was not encountered.

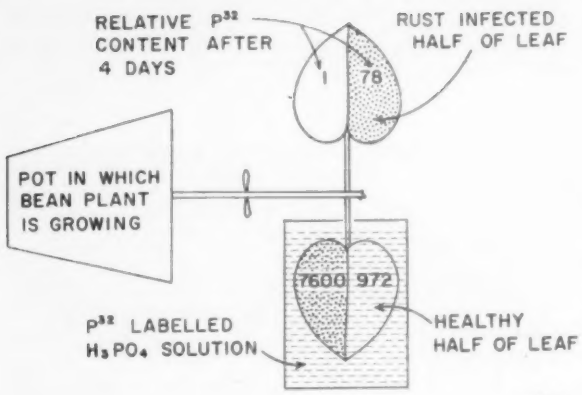
RESULTS.—*Direct applications.—Nicotiana glutinosa* leaves were inoculated with tobacco mosaic virus, exposed to vapors from Na_2S^{35} solutions, and autoradiographed. These leaves gave the sharpest autoradiographs secured. Leaves infected for only 1 day showed no selective accumulation. Leaves infected for 2 days showed localized accumulation of S^{35} before any lesions were apparent to the eye. Leaves infected for 3 or more days showed marked selective accumulation (Fig. 1) in the virus lesions. This could indicate accumulation of S by the virus. However, leaves of tobacco (*Nicotiana tabacum*) systemically infected with tobacco mosaic virus did not absorb more S^{35} than did healthy leaves. It is tentatively concluded that the selective accumulation of S by tobacco mosaic virus local lesions on *N. glutinosa* is a result of necrotic leaf injury, but this explanation is only partially satisfactory. Injury to leaves by various degrees of mechanical pressure up to and including killing of the treated area, or injury with drops of a range of concentrations of H_2SO_4 up to killing dosages did cause increased accumulation of S wherever injury occurred, but in no case was the magnitude of selective accumulation as great as that occurring around local lesions caused by tobacco mosaic virus.

Pinto bean leaves inoculated with rust, exposed to vapor from Na_2S^{35} and autoradiographed, showed accumulation of S in the rusted areas (Fig. 2, 4–8). Washing of the leaves with a mild detergent before printing caused no detectable alteration of the autoradiographs. This indicated that the labelled S was firmly bound to the leaf. Selective accumulation increased with age of infection. At 4 days after inoculation, the rust infections were not visible by direct visual observation but were apparent in the autoradiograph as dark circles of low radioactivity (low sulfur accumulation) while the centers of the infections showed about the same accumulation of sulfur as the older infected leaf tissue. At 6 days after inoculation (Fig. 4) 3 zones of sulfur accumulation were apparent at the area of infection. The center region showed low sulfur accumulation, beyond this was a region of greatest accumulation of sulfur, and beyond this was a wide zone of distinctly lower sulfur accumulation than in the unaffected portion of the leaf. At 8 days after inoculation (Fig. 5) 4 zones of sulfur accumulation were discernible at the rust pustule. Here, contrary to the situation at 4 and 6 days after inoculation, the region of highest concentration was the center of the rust pustule. The outside diameters of the 4 zones were approximately 0.5, 0.8, 1.8, and 3.5 mm. Clearly defined accumulation zones were usually less apparent in the older infections (Fig. 6, 7), and only 1 zone of sulfur accumulation was apparent in a 12-day infection where the rust pustules were close together (Fig. 7), but for unknown reasons, there was considerable variation among trials. Even with 8-day pustules (Fig. 5, 8) the central region of the pustule was sometimes a zone of heavy accumulation and sometimes a region of low accumulation. Healthy leaves showed no localized accumulations of S (Fig. 3).

FIG. 1.–10. Fig. 1–8 and 10 are autoradiographs of leaves in which the pale areas indicate high radioactivity.—Fig. 1. H_2S^{35}-treated Nicotiana glutinosa leaf. The leaf was exposed to vapors from Na_2S^{35} for 8 hours 5 days after inoculation with tobacco mosaic virus. The position of the white areas corresponds to the position of the virus local lesions. In determinations in a Geiger counter, the virus lesions contained 19 times as much S^{35} per unit area as the healthy areas of the leaf.—Fig. 2. Bean leaf which was exposed to vapor from Na_2S^{35} for 8 hours at 7 days after inoculation with rust Uromyces phaseoli. The pale areas correspond in position to the rust lesions.—Fig. 3. Healthy bean leaf which was exposed to Na_2S^{35}. The leaf shows uniform distribution of S^{35} except for the low accumulation along the veins and the high accumulation at the margins.—Fig. 4–8. Bean leaves which were exposed to Na_2S^{35} at various times after inoculation with rust. Fig. 4, 6 days after inoculation; Fig. 5, 8 days after inoculation; Fig. 6, 20 days after inoculation; Fig. 7, 12 days after inoculation; Fig. 8, 8 days after inoculation (see also fig. 5). The pale circular areas correspond to the position of the rust pustules.—Fig. 9. Rusted bean leaf showing starch accumulation around pustules. The leaf was placed in hot water 11 days after inoculation, then bleached in alcohol, stained in IKI, and partially cleared in chloral hydrate.—Fig. 10. Autoradiograph of upper surface of bean leaf exposed to Na_2S^{35} 10 days after inoculation with mildew (Erysiphe polygoni). The diffuse areas of high accumulation of radioactivity correspond to the position of the mildew colonies.

The apparent similarity in the accumulation of starch (Fig. 9) and of sulfur is noteworthy. There were as many as 3 zones of starch accumulation in well isolated pustules (lower center, Fig. 9) or starch accumulation was absent or nearly so in crowded pustules (lower left, Fig. 9).





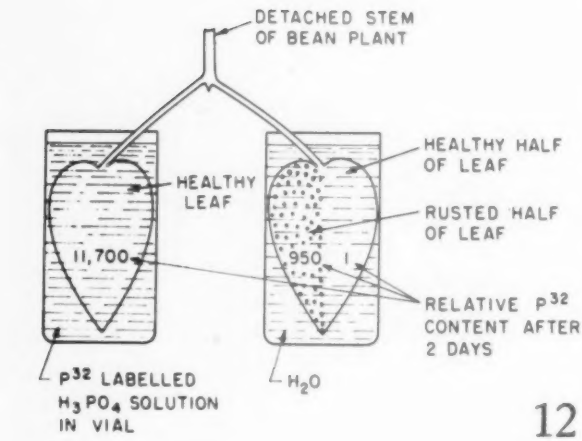


FIG. 11–12.—Fig. 11. Schematic diagram of uptake and translocation experiments with bean plants. Partially rusted bean leaf immersed in radioactive solution, opposite partially rusted leaf exposed to air.—Fig. 12. Healthy bean leaf immersed in radioactive solution, opposite partially rusted leaf immersed in water. The numbers represent relative P^{32} contents obtained in a typical experiment with labeled H_3PO_4 solution.

Powdery mildew infections showed less distinct autoradiographs than rust or virus infections. Figure 10 is the clearest autoradiograph secured of bean powdery mildew, but the true radiating character of the mildew colonies does not appear in the autoradiograph. This may be due to the fact that sulfur is accumulated by the injured host cells rather than by the mildew mycelium. Occasionally an erratic S^{35} uptake occurred on the upper leaf surface which could not be correlated with known surface infections.

Direct measurement of activity with a GM counter was used to measure the uptake of S^{35}, P^{32} and C^{14} (as H_2S, H_3PO_4 and sucrose) by diseased leaf tissue. A greater uptake of S^{35} by diseased tissue than by comparable healthy tissue occurred in the following cases: *Phaseolus vulgaris* infected with *Uromyces phaseoli*, *Antirrhinum majus* with *Puccinia antirrhinum*, *Bromus* sp. with *Puccinia rubigo-vera*, *Solidago californica* with *Uromyces perigynius*, *Cucumis sativus* with *Erysiphe cichoracearum*, *Phaseolus vulgaris* with *Erysiphe polygoni*, *Prunus persica* with *Taphrina deformans*, *Vicia faba* with *Ascochyta pisi*, *Pyrus communis* with *Venturia pyrina*, *Vitis californica* with *Plasmopara viticola*, *Humulus lupulus* with *Pseudoperonospora humuli*, *Hordeum vulgare* with *Pyrenophora teres*, *Iris* sp. with *Heterosporium gracile*, *Aesculus californica* with *Septoria aesculi*, *Sonchus asper* with *Septoria lactucae*, *Phaseolus vulgaris* with tobacco mosaic virus, and *Nicotiana glutinosa* with tobacco mosaic virus.

The ratios of accumulation (i.e., the ratio of activities in the diseased and healthy tissues) observed in the above materials varied from 1.1:1–19:1. This latter value was found with 5-day old infections of tobacco mosaic virus on *N. glutinosa* exposed to labeled H_2S vapor.

The following diseased leaf tissues showed less accumulation of S^{35} or P^{32} than comparable healthy tissues: *Pyrus malus* infected with *Podosphaera leucotricha*, *Quercus agrifolia* with *Sphaerotheca lanestris*, *Rosa* sp. with *S. pannosa*, *Brassica compestris* with *Albugo candida*, and *Clematis* sp. with *Puccinia rubigo-vera*. The lowest accumulation ratio, 0.35:1, was obtained with labelled H_2S treatment of natural aecial infections of *Puccinia rubigo-vera* of *Clematis*.

The following diseased leaf tissues did not reveal clear differences of accumulation between diseased and healthy tissues: *Nicotiana tabacum* infected with tobacco mosaic virus (S^{35}), *Rubus ursinus* with *Peronospora rubi* (S^{35}), *Calendula* sp. with *Erysiphe cichoracearum* (S^{35}), *Phaseolus vulgaris* with *Colletotrichum lindemuthianum* (C^{14}), *Rumex crispus* with *Ovularia* sp. (C^{14} and S^{35}) and *Gardenia* sp. with *Xanthomonas maculifolia* (C^{14}).

Accumulation via translocation.—In the experiments to be described, the test substances were introduced into the plant and their subsequent distribution determined. Results of 2 experiments in which one leaf was immersed in P^{32} labeled H_3PO_4 or C^{14} labeled sucrose and the opposite leaf exposed to air (Fig. 11) are in Table 1. These experiments were conducted in the dark and the immersed leaf contained more P^{32} or C^{14} than the opposite non-immersed leaf. However, in a similar experiment conducted in natural light in the greenhouse for 3 days, more P^{32} accumulated in the aerial than in the immersed leaf. The data of Table 1 indicate that greater differences in concentration between healthy and rusted tissues occurred with P^{32} than with C^{14} in both immersed and aerial leaves. Removing the root from the plant (i.e., detached plant) had little effect on the distribution of C^{14}. With P^{32}, the greatest differences in concentration were obtained with detached plants. In Table 1 the

TABLE 1.—*Selective accumulation of carbon and phosphorus by rusted and healthy tissues of attached and detached bean plants (Fig. 11)*[a]

	Counts/sec./cm²				
	C^{14} supplied as C^{14}-sucrose		P^{32} supplied as $H_3P^{32}O_4$		
	Attached plant 63 hr.	Detached plant 63 hr.	Attached plant 19 hr.	Attached plant 91 hr.	Detached plant 91 hr.
Leaf in test solution					
healthy tissue	1.50	1.10	122	1200	966
rusted tissue	4.63	4.04	404	9420	11800
Opposite leaf in air					
healthy tissue	0.072	0.027	0.57	1.24	0.64
rusted tissue	1.15	0.40	1.35	96.6	422.

[a] Half-rusted leaf immersed in 30 ml of radioactive solution (1% sucrose, 0.1/μc/ml or very high specific activity H_3PO_4, 1/μc/ml) for indicated periods at room temperature in the dark. Opposite half-rusted leaf not treated.

maximum accumulation ratio was 690:1 (opposite leaf in air, detached plant, 91 hr.), but in another experiment where the immersed leaf was healthy and ½ of the non-immersed leaf had been inoculated with rust 5 days before the start of the test, a ratio of 1600 was measured.

When one leaf was immersed in the labeled phosphate solution and the opposite leaf was in water (Fig. 12) much less total P^{32} was translocated from the phosphate treated to the opposite leaf than when the opposite leaf was not immersed. It was further observed that with both leaves immersed, greater translocation occurred if the leaf in the phosphate solution was healthy. Also the P^{32} ratio of the rusted to the healthy tissues of the leaf in water was greater in this case. Treating a partially or completely rusted leaf with the radioactive solution resulted in reduced values of translocation. However, if the leaf immersed in the water were wholly or partly rusted, more P^{32} moved into it than if it were healthy. In general, selective accumulation of P^{32} was not clearly related to its total translocation.

The largest P^{32} ratio of rusted to healthy tissue observed was 7870:1. This was obtained with a bean plant heavily inoculated with rust on one side of the midrib of one leaf. Five days after inoculation the plant stem was severed above the cotyledonary node. The healthy leaf was immersed in the labeled phosphate solution, and the opposite half-rusted leaf immersed in water (Fig. 12). After 2 days in darkness, the leaf in water was removed and washed and 6 cm² discs were cut from the healthy and rusted sides of the leaf, dried, and assayed for radioactivity.

In other similar experiments (healthy leaf in P^{32} solution, opposite half-rusted leaf in water) ratios varying from 96:1–1645:1 were obtained. These experiments involved different intensities of rust infection, different intervals from inoculation to start of tests, different times of start of test and different durations of test. At the present time, the relative importance of the above factors cannot be adequately appraised.

More P^{32} or C^{14} accumulated in rusted than in comparable healthy plants, leaves, or half leaves. The accumulation ratios were greater when the rusted and healthy areas were on the same leaf, but on opposite sides of the midrib, than when on opposite leaves of the same plant or on different plants. When labeled phosphate was applied to the soil, greater total accumulation occurred in light than in darkness, but greater accumulation ratios resulted in darkness. Much larger accumulation ratios resulted when the P^{32} or C^{14} was absorbed by the opposite leaf than when absorbed by the roots or cut stems.

Selective accumulation occurred even when the rust mycelium was killed. Half rusted leaves were heated for 100 seconds at 45°C to kill the rust (7) but not the leaf tissue, and then used in translocation experiments as in Figure 12 in comparison with rusted plants in which the rust had not been killed. In one trial the counts/cm²/second over background averaged 223 for the healthy leaves immersed in $H_3P^{32}O_4$; 0.044 and 97 for the healthy and infected portions respectively of a leaf with a living 3 day rust; 0.028 and 0.028 for a leaf with killed 3 day rust; 0.5 and 227 for living 5 day rust; 0.05 and 49 for killed 5 day rust; 0.028 and 34.1 for living 7 day rust; and 0.00 and 6.5 for killed 7 day rust. In all cases, there was less accumulation of P^{32} in rusted areas in which the rust had been killed than in comparable areas of living rust. In all cases except the killed 3 day rust, there was much greater accumulation of P^{32} in dead rust areas than in non-rusted halves of the same leaves. No P^{32} was detected in the water (Fig. 12) in which the half rusted leaves were immersed in the above tests.

DISCUSSION.—The simplest explanation for the observed greater accumulation of chemicals by diseased than by healthy tissues would be that a greater uptake of the chemical occurs in the pathogen than in the host. Gottlieb and Garner (1) reported less P^{32} in rust spores of treated wheat leaves than in the non-rusted portions of the leaves, whereas Yarwood and Jacobson (10) found a greater accumulation of S in rust spores than in non-rusted bean leaves. In neither case is it valid to apply to the mycelium within the leaves the results secured with spores.

The high accumulation of S^{35} in mildewed areas on bean leaves (9 and this paper) and low accumulation in the fungus (10) suggest that the selective accumulation must be in the diseased host tissue. This is contrary to the conclusion of Miller, McCallan and Weed (3) but in agreement with the conclusion of Shaw, Brown, and Jones (4).

A possible explanation of the observed selective accumulation could be the greater permeability of diseased than normal tissues (6). It might be expected that those infections which caused the greatest

injury would also cause the greatest ease of entry of chemicals. Swanson and Whitney (5) observed that the uptake of P^{32} was greatly increased when the $H_3P^{32}O_4$ was applied at pH values low enough to cause slight necrosis. The greater accumulation ratio in tobacco mosaic virus-infected *Nicotiana glutinosa* (necrotic lesions) than in *Nicotiana tabacum* (no necrosis) would support the view that injury favors accumulation. On the other hand, rusts as a group and bean rust in particular probably caused less necrosis than any other group of pathogens studied, yet they showed greater selective accumulation than necrotic infections such as those caused by *Colletotrichum, Pyrenophora, Ramularia, Ovularia,* and *Xanthomonas.* Shaw, Brown, and Jones (4) found that injury reduced accumulation and interpreted their selective accumulation as principally due to translocation.

Rupture of the leaf epidermis during sporulation of the pathogen might be expected to facilitate the penetration by chemicals, and undoubtedly played a part in selective accumulation in some cases. With bean rust, selective accumulation increased with age of infections and the epidermis was usually ruptured in about 8 days after inoculation. However, selective accumulation was clearly demonstrated before rupture of the epidermis occurred, and there was no apparent sudden increase in selective accumulation associated with the rupturing of the epidermis. In one case where a leaf, treated with labeled H_2S, bore both open and unopened pustules, there was no clear difference in the uptake of the labeled H_2S by open and unopened pustules. Also the greatest selective accumulation resulted in translocation tests (Fig. 12) where any direct effect of rust infection on stomatal opening could hardly affect accumulation.

The greater accumulation of chemicals at infection spots on leaves (2) has been offered as evidence that infection spots transpire more rapidly than normal tissues. Greater localized transpiration might then be a means of bringing about selective accumulation. However no transpiration data are presented by Harvey (2), and accumulation of chemicals at infection spots could be brought about by causes other than increased transpiration. In most of the present trials, experiments were carried out in closed petri dishes and pint jars or with immersed leaves, and transpiration was nil or very low.

There is no obvious relation between the nutritive or toxic value of chemicals and their selective accumulation (10). Sucrose and very dilute H_3PO_4 could be considered nutritive, and H_2S toxic. Yet all were selectively accumulated under certain conditions.

No specific evidence as to whether the accumulated chemicals were localized in the interior or on the surface of the leaf was obtained in most experiments. However, the facts that the internal rust fungus was killed by gaseous sulfur treatments (7), that washing H_2S^{35}-exposed leaves caused no alteration in the distribution pattern of radioactivity, and that the uptake of P^{32} and C^{14} by the rusted tissue in the translocation trials was only from the translocation system, indicate that the accumulation was primarily in the interior of the leaf.

The remarkably high accumulation ratios obtained in the P^{32} translocation experiments could be explained in part on the basis of the high metabolic activity of the diseased tissue. The greater respiration rate of rusted than normal tissues even when the rust is killed (8) could account for the high accumulation of P in these tissues. The zones of varying radioactivity observed about the rust pustules in the autoradiographs of S^{35} treated bean leaves could represent varying intensities of metabolic activity.

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THE ELECTRON MICROSCOPY OF A BACTERIOPHAGE ATTACKING XANTHOMONAS MALVACEARUM [1]

David W. Rosberg and A. L. Parrack [2]

SUMMARY

A bacteriophage that attacks *X. malvacearum* was isolated from 2-year-old dried bacterial blight-diseased cotton leaves. Attempts to isolate a bacteriophage from fresh leaves of greenhouse and field grown blight-infected cotton plants were unsuccessful.

The bacteriophage isolated is stable in culture at room temperature and resists desiccation when associated with its bacterial host in cotton leaf tissue for at least 48 months. The bacteriophage particles are spherical, about 27 mμ in diameter and without distinct tails.

Electron microscope studies of bacteriophage-reacted 24-hour-old cultures of *X. malvacearum* indicate that adsorption of bacteriophage particles on the bacterial cells occurs in 30–45 minutes. After 4 hours, approximately 90 per cent of the cells are lysed or burst, as characterized by a disintegrating diffuse bacterial body and greatly decreased electron density.

The granular periphery (shell) of lysed bacteria apparently is not utilized in bacteriophage reduplication during a reaction period of 4 hours. Until 12 days elapsed, no bacterial growth was observed when bacteriophage-reacted cultures were poured onto the surface of potato-dextrose agar medium.

It is possible that bacteriophage isolates will prove useful in identifying strains of *X. malvacearum* that occur throughout the cotton belt.

A number of bacteriophages that attack specific bacterial plant pathogens have been reported. Thomas (5) and Thornberry, Braun, and Elrod (6) have demonstrated that bacteriophages can be used effectively to identify phytopathogenic bacteria.

This study concerns a bacteriophage not previously reported to have been isolated in the United States, which attacks *Xanthomonas malvacearum* (E. F. Smith) Dowson, the causal agent of bacterial blight of cotton. Massey (1, 2) obtained a bacteriophage against *X. malvacearum* from Blue Nile River water and from soil filtrates from fields of blight-infected cotton which had been overflowed by the Blue Nile. Matsumoto and Huzioka (3), working in Japan, isolated a bacteriophage from blight-infected cotton leaves and demonstrated bacteriophage resistance in 4 strains of *X. malvacearum* isolated from bacteriophage-reacted cultures. Okabe (4) obtained 32 colony variants from 2 strains of *X. malvacearum* isolated from cotton in Formosa. He found all 23 variants of one strain to be susceptible to bacteriophage and the 9 variants of the other strain bacteriophage resistant. Okabe attributed this differential reaction to the presence of at least 2 elemental bacteriophages in the bacteriophage filtrate used. The electron microscopy of *X. malvacearum* bacteriophage also has not been reported.

MATERIALS AND METHODS.—Filtrates of fresh and dry blight-infected cotton leaves were used as the bacteriophage source. One hundred g. of fresh leaves were crushed and finely chopped and placed in a 1000 ml Erlenmeyer flask. Approximately the same quantity of 2-year old dried, diseased leaves collected the summer of 1950 were similarly prepared. One hundred fifty ml of distilled water were added to each flask of leaf material and thoroughly shaken. The flasks were placed in a refrigerator, held at 8–10°C. for 3 days and shaken vigorously twice each day. The liquid (steep) from each flask was decanted, passed through a Buchner filter and then through a Seitz bacterial filter. The filtrates thus obtained were used against nutrient broth cultures of *X. malvacearum*, (24 hours growth in 10 ml broth).

In the initial test for the detection of the presence of bacteriophage, 0.5 ml of fresh and dried leaf steep were carefully overlayered in each of 2 sets of 10 nutrient broth cultures of *X. malvacearum*. After an additional 24 hours incubation at room temperature the 10 culture tubes that received 0.5 ml of steep from dried 2-year-old infected cotton leaves showed definite lysis in the upper ⅓ of each culture tube. No apparent bacterial lysis occurred in the second set of 10 cultures to which the same quantity of fresh cotton leaf steep had been added.

In a second series, 1 each of the dried and fresh-leaf-reacted cultures were centrifuged and the supernatant passed through a Seitz filter; 0.5 ml of filtrate were again carefully overlayered to 2 sets of ten 24-hour nutrient broth cultures of *X. malvacearum* and stored at room temperature. At the end of 20 hours, all 10 cultures that received 0.5 ml of the filtrate from dried-leaf-steep-reacted cultures were completely lysed. There was no recurrence of bacterial growth in the cultures for 12 days, after which bacteria were recovered when lysed cultures were poured onto the surface of potato-dextrose culture media. There was no apparent bacteriophage activity in any of the cultures that received the supernatant from the initial fresh-leaf-steep-reacted cultures after 10 serial passages

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through 24-hour cultures of *X. malvacearum*. A later attempt to isolate a bacteriophage from fresh leaves of naturally infected cotton plants was also unsuccessful.

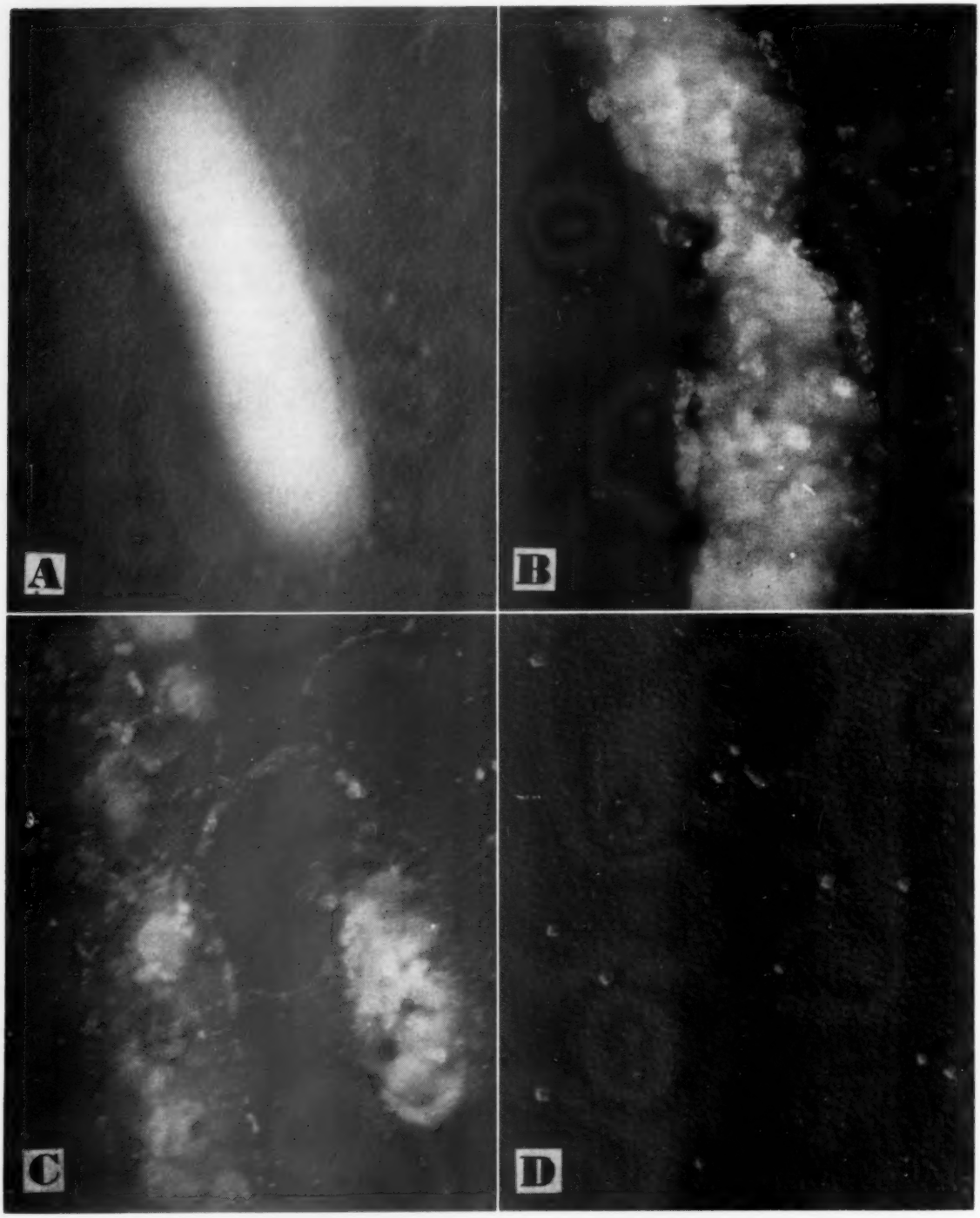

FIG. 1. Electron micrographs of the lytic action of *X. malvacearum* bacteriophage. A) 30-minute reaction showing adsorbed bacteriophage particles on a single bacterium (× 26,000). B) Interrupted fission of a bacterium due to lytic process of bacteriophage. After 2 hours, the interior of the cell has a lower electron density revealing its granular nature (× 26,000). C) Four hour reaction. The granular periphery (shell) of a lysed bacterium (center). The undivided bacterium at left is less granular and its electron density further reduced (× 26,000). D) Unadsorbed bacteriophage particles (× 54,000).

ELECTRON MICROSCOPE STUDY.—To a series of 10 24-hour old nutrient broth cultures of *X. malvacearum*, 0.2 ml of bacteriophage-containing filtrate was added. At intervals of 15 minutes for the first hour and 30 minutes for an additional 3 hours, microdrops from bacteriophage-reacted cultures were mounted on electron microscope viewing screens. These cultures had been centrifuged at 2400 rpm and the bacterial precipitate washed and re-suspended in a volume of 2.0 ml of 85 per cent NaCl. This treatment was necessary to remove constituents of the culture medium that "boiled" on the viewing screens in the electron microscope, obliterating the bacteria and bacteriophage. The screens had been coated with a Formvar film prepared from 0.2 per cent Formvar dissolved in ethylene-dichloride. The viewing screens were gold-shadow-casted in an RCA EMV-6 Shadow Unit and examined with an RCA Type EMU electron microscope.

RESULTS.—The earliest evidence of adsorption of bacteriophage particles on the bacterial cells appeared in the 30-minute and 45-minute bacteriophage-reacted cultures. The number of bacteriophage particles surrounding the individual bacterial cells varied from 2–18 (Fig. 1, A). In every case, spherical bacteriophage particles were observed attached to the bacterial cells. The first evidence of lytic action on the bacterial cells appeared in 1-hour bacteriophage-reacted cultures. The affected bacteria were enlarged and a coarsely granular periphery approximately 60 mμ in diameter, became delimited from the interior of the cell. This peripheral material remained intact or disintegrated when completely delimited (Fig. 1, B and C). In the screens examined, the number of lysed bacterial cells per screen increased with time, i.e., bacteriophage-bacterium association. In 4-hour bacteriophage-reacted preparations, approximately 90 per cent of the bacterial cells were completely lysed or showed evidence of lytic action characterized by a disintegrating diffuse cell and greatly decreased electron density (Fig. 1, C). The bacteriophage particles appeared spherical in shape without visible tails and were about 27 mμ in diameter (Fig. 1, D). There was a suggestion of a tail on some non-adsorbed bacteriophage particles in 30 and 45 minute preparations, but these protuberances were not uniform and their size difficult to calculate. The individual bacteriophage particles were not distinguishable on or within the bacterial cells beyond the initial adsorption phase of the lytic process.

DISCUSSION.—In consideration of the fact that there was no recurrence of bacterial growth for 12 days in lysed cultures, nor when these cultures were poured onto potato-dextrose culture media, it is possible that the bacteriophage isolated was actually a mixture of bacteriophages capable of lysing all forms of the bacterium including bacteriophage-resistant mutants which usually arise in bacteriophage-reacted bacterial cultures. Since the bacteriophage, or bacteriophage mixture herein reported, that attacks *X. malvacearum* was isolated from dried 2-year-old bacterial blight diseased leaves, it is assumed to be reasonably stable and unaffected by relatively long periods of desiccation. Attempts to isolate a bacteriophage against *X. malvacearum* from both artificially and naturally-infected fresh cotton leaves were unsuccessful.

Electron microscope studies of lysed bacterial cells indicate that the granular periphery (shell) of lysed cells, which appeared intact in considerable numbers in 4-hour reacted preparations, is not utilized in bacteriophage re-duplication during the 4-hour reaction period. Thornberry et al observed similar bacterial shells remaining after bacteriophage lysis of *Xanthomonas pruni* (6). The apparent disintegrated peripheral (shell) material of many bacteria in various stages of lysis probably was due to the effects of centrifugation and washing in the preparation of cultures for electron microscopy.

The effect of bacteriophage as a factor in limiting the severity of bacterial blight disease in field-grown cotton has been studied by Massey (2), who found the incidence of bacterial blight disease reduced in fields that had been overflowed by the Blue Nile River. Massey attributed this effect to destruction of *X. malvacearum* in the soil by bacteriophage carried in the Blue Nile River water. In view of this information, the application of concentrated phage as a practical control measure to prevent infection of cotton may have some merit in sections where cotton is grown under irrigated conditions.

Although many difficulties undoubtedly will be encountered in attempts at control of the bacterial blight bacterium by bacteriophage in the field, preliminary studies of isolates of *X. malvacearum* from North Texas and New Mexico indicate that the bacteriophage isolated may be a useful tool in identifying strains of *X. malvacearum* occurring throughout the cotton belt.

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TRANSMISSION OF FIG MOSAIC BY THE ERIOPHYID MITE ACERIA FICUS[1]

R. A. Flock and J. M. Wallace

SUMMARY

Fig mosaic was shown to be transmitted by the eriophyid mite *Aceria ficus* (Cotte), and was shown not to be the result of toxicogenic feeding. Non-viruliferous mites were found to be highly destructive to fig trees but they did not cause typical mosaic symptoms. Fig mosaic was found to be present on all fig trees examined in the field in California. Measurement of loss of fruit production caused by the disease has not been possible because of lack of healthy plants other than seedlings for comparison.

Mosaic-like symptoms commonly occur on fig trees in California. Field symptoms include chlorotic mottling and spotting of the foliage and extensive leaf distortion.

Fig mosaic was described as a virus disease by Condit and Horne (4). These authors observed the fig mite and several insects on the fig and suspected the mite of transmitting fig mosaic. On the basis of the appearance of mosaic-like symptoms on new growth from buds of symptomless, mite-free seedlings grafted into diseased orchard trees, they concluded that the causal agent of fig mosaic was graft transmissible. They observed the prevalence of the fig mite and attributed certain injuries to the mite infestations, suggesting that the leaf-spotting might possibly result from "localized feeding of the mites when the leaves were young and tender."

In view of the fact that the graft-transmission tests of Condit and Horne (4) were made on mite-infested trees, the results obtained were not conclusive proof of graft transmission. If the mosaic-like symptoms were simply a toxicogenic reaction, the symptoms that developed on the growth from the healthy buds could have resulted likewise from direct feeding of the mites.

Condit and Horne (4) reported, also, that in 2 instances at least, healthy fig seedlings developed mosaic symptoms after having mite-infested fig leaves placed on them. Such results were suggestive of mite transmission but could not be accepted as conclusive proof inasmuch as the plants were not shown to continue to display mosaic symptoms when freed of mites.

In 1944, symptoms that appeared to be true mosaic were obtained by Wallace (9) in tests similar to those made by Condit and Horne. Mite-infested bud scales and young leaves from field fig trees were placed on small, caged fig seedlings. A number of the test seedlings later developed symptoms that were diagnosed as true mosaic. However, inasmuch as these seedlings were not treated to eliminate the mites, the possibility remained that the symptoms observed could have resulted from the continuous presence of fig mites.

Studies on the relationship of the fig mite, *Aceria ficus* (Cotte), to the fig mosaic disease were renewed in 1951. This paper reports the results obtained in these investigations.

[1] Accepted for publication October 4, 1954.
Paper No. 825, University of California Citrus Experiment Station, Riverside, California.

THE FIG MITE.—The fig mite was first reported in California as *Eriophyes fici* M. S. Ewing, by Essig (6) and was first described in detail by Keifer (7) under the name *Eriophyes fici* Essig. Baker (2) placed the California species under *Aceria ficus* (Cotte), which was described from France (5).

The fig mite, *Aceria ficus* (Cotte) (Fig. 1), is a small mite, ranging from 1/125 to 1/200 of an inch in length. It is pale yellow in color, is wider anteriorly than posteriorly, and has 2 pairs of legs. It is usually found in the buds and on young leaves, but it may occur in huge numbers on old leaves and inside fruit. Feeding may cause a russeting or browning of the plant surface. The mite can cause a blasting of the buds and a chlorosis of the leaves and defoliation of branches or whole trees. In the greenhouse, new growth may be entirely prevented by the presence of this mite. In the field, mosaic and leaf distortion symptoms are commonly associated with numbers of this mite. At least part of these symptoms resemble the effects on other plants demonstrated to be caused by the feeding of eriophyid mites. A good example of mosaic-like symptoms caused by a toxicogenic mite is yellow spot of peaches (10).

EXPERIMENTAL METHODS AND RESULTS. — *Mosaic symtoms distinct from feeding injury.*—Experiments were set up to show whether or not mosaic symptoms would persist on fig plants in the absence of fig mite infestations. A series of 37 cuttings from field trees showing mosaic symptoms were treated with dusting sulfur to kill fig mites. The cuttings were then rooted and grown in a mite-free environment. Mosaic symptoms eventually appeared on new growth on these cuttings. In other tests, 5 fig seedlings became infected and developed mosaic symptoms after having scions grafted into them from diseased, mite-free sources.

These tests demonstrated satisfactorily that the mosaic-like symptoms on figs did not result entirely from the toxic effect of mite feeding. The transmission by grafting gave good evidence that fig mosaic is caused by a transmissible virus.

Transmission of the fig mosaic virus by the fig mite.—Mites from mosaic-infected fig trees were transferred individually with a fine brush to small seedling fig plants that were kept in mite-free cages. The test plants were dusted with sulfur after 3 to 5 days. The first tests were made with a large number of mites on a few test plants. Some mosaic-like symptoms devel-

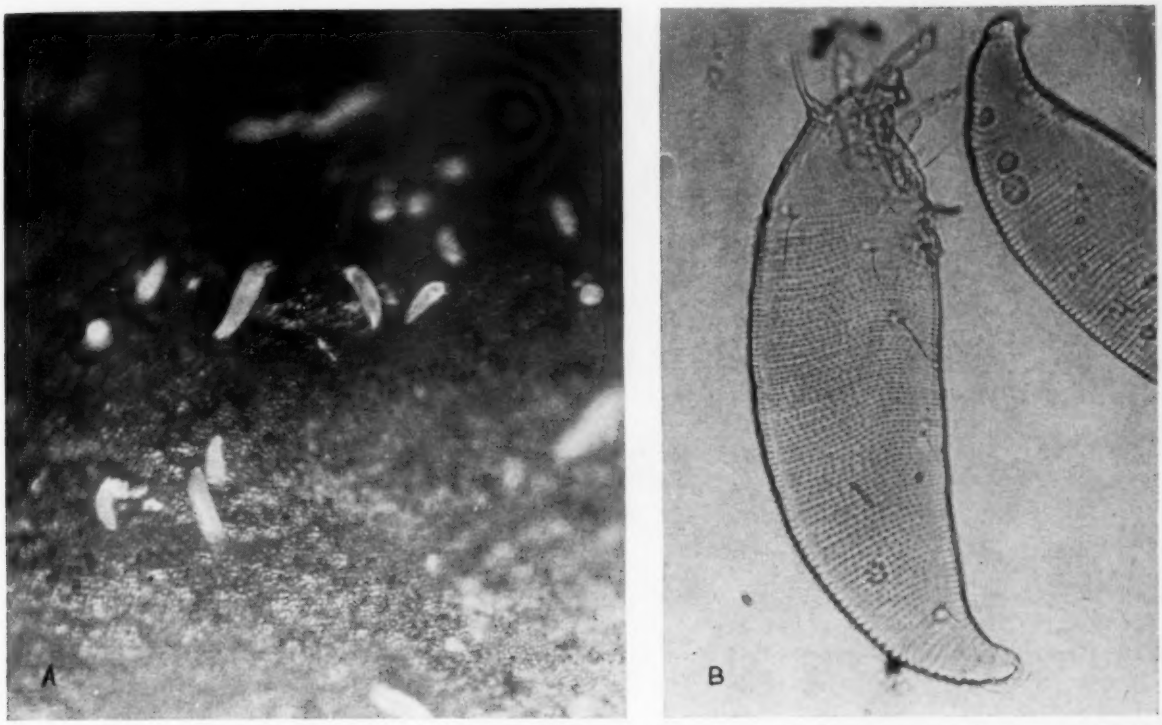

FIG. 1. Fig mite, *Aceria ficus* (Cotte). A) Various stages and eggs on leaf. B) Cleared, mounted specimen showing sculpture and setae (photographed by Norton Wilson, U.S.D.A.).

oped in less than 10 days. Because of the short time required for these effects to appear, it seemed likely that these early symptoms resulted from the toxic effect of mite feeding. Some of the plants appeared to recover partially but later developed more severe symptoms. These reactions suggested that the first symptoms to appear may have been a toxicogenic reaction. Later tests were made with smaller numbers of mites, and a good percentage of transmission was obtained with all of them. These data are summarized in Table 1.

To differentiate clearly between symptoms resulting directly from the feeding of the mites and the true virus mosaic effects, a virus-free colony of mites has been developed. To get such a colony, mites have been grown from eggs transferred to disease-free seedlings. Fourteen plants exposed to the feeding of disease-free mites for 5 days have shown leaf distortion and slight chlorosis and russetting but no mosaic symptoms.

In a later test, 7 plants on which virus-free mites were grown, and 8 plants on which an average of 133 mites were established, have shown no mosaic symptoms. The 8 plants were fumigated 2 weeks after the mites were applied and were reinfested at 1, 2, and 3 months. The plants were then kept free from mites for 2 additional months.

Transmission tests with other insects.—Surveys were made in late 1951 and 1952 to determine what other potential virus vectors were to be found on the fig. The phytoseiid mites *Typhlodromus longipilus* Nesbitt and *Typhlodromus* sp. (Det. Baker) were the most abundant. Tetranychid mites were found only occasionally in southern California. Flower thrips, *Frankliniella* sp., and the aphids *Aphis gossypii* Glover and *Myzus persicae* (Sulz.) were present in moderate numbers. The fig psyllid *Homotoma ficus* L., which Castellani (3) reported as the probable vector or cause of fig mosaic in Italy, has not been found in California.

A series of transmission tests to 5 plants each was made with these species. No evidence was obtained that any of these insects are vectors of fig mosaic.

Symptomatology of fig mosaic.—Symptoms of fig mosaic are very erratic in occurrence. Some of the inoculated plants have shown only one small chlorotic spot for months before developing conspicuous markings. Symptoms developed more consistently at 90°F than at 80°F.

Symptoms on the test plants were highly variable (Fig. 2). Some markings consisted of faint, diffuse

TABLE 1.—*Transmission of fig mosaic virus by the fig mite Aceria ficus (Cotte)*

Number of mites per plant	Number of plants	Percentage of plants showing symptoms Within 10 days	Within 90 days
200	6	100	100
100	20	55	90
50	19	57.9	94.7
20	19	21.1	84.2
5	15	40	93.2
1	10	20	70
0	67	0	0

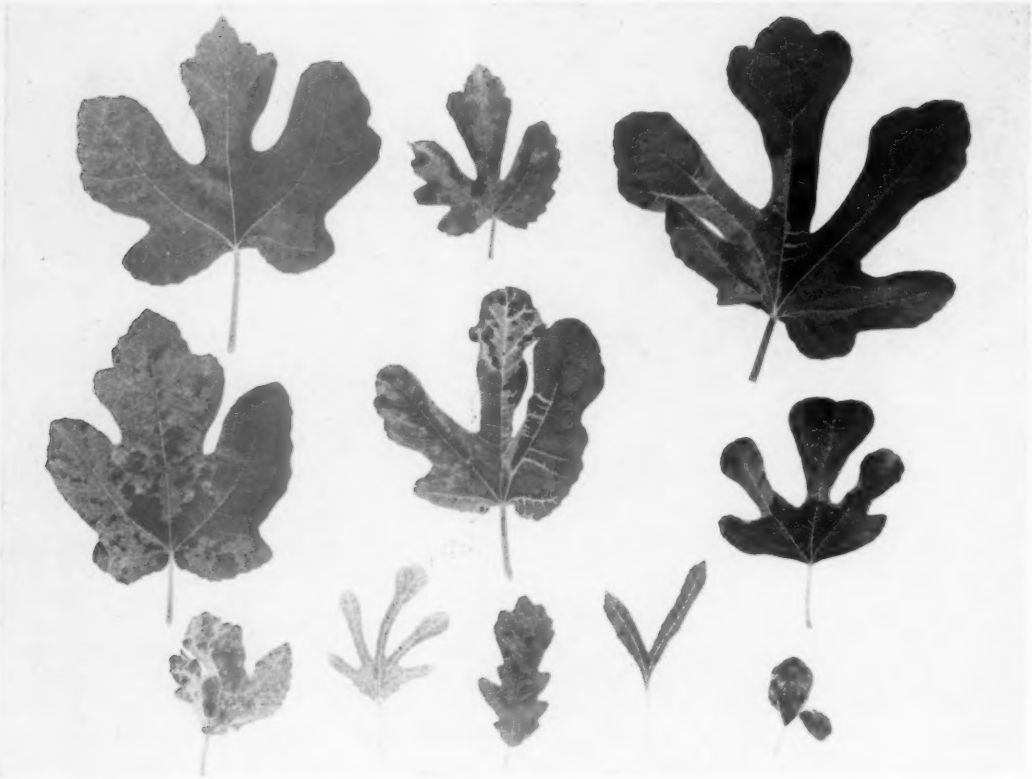

FIG. 2. Characteristic patterns and distortion of fig leaves from mosaic-diseased test plants.

areas without clear-cut margins, whereas others were well delineated and contrasted sharply with the leaf in color. The markings varied greatly in size and shape. Many of the leaves showed marked distortion.

DISCUSSION.—Few plant viruses have been shown to be transmitted by mites. Amos *et al.* (1), in England, presented inconclusive evidence of transmission of the currant reversion virus by the currant big bud mite. Massee (8), using approximately 7200 mites per plant, produced reversion symptoms on 25 test plants, but he does not entirely rule out the toxicogenic factor of mite feeding. Slykhuis (11) reports transmission of wheat streak mosaic by *Aceria tulipae* Keifer. Fig mosaic appears to be the first tree virus demonstrated to be transmitted by a mite.

Demonstration of transmission of fig mosaic by the fig mite opens up some interesting possibilities in fig culture. It will be important to determine whether or not the fig mite and fig mosaic can be controlled economically in the field. Successful control of the fig mite and of the injury it causes by direct feeding might make fig growing more successful in southern California. It will be necessary to study mite control on isolated fig plantings started with mosaic-free plants to determine the possibility and benefits of field control of fig mosaic. This will necessitate the finding of virus-free fig varieties from which propagations can be made. No such material is available locally.

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Entomosporium leaf spot of Rhaphiolepis. ROBERT D. RAABE AND H. N. HANSEN. During the spring of 1954, a serious leaf spot disease was found on India-Hawthorn, *Rhaphiolepis indica* Lindl., in several nurseries in the San Francisco Bay area. Symptoms appeared as small, circular, slightly elevated spots, gray or tan in color and varying in size from 1–8 mm in diameter. Tissues surrounding the lesions were frequently brown or purple and appeared as halo spots. Tissue on the upper surface of the leaf sometimes became chlorotic, thereby adding to the halo effect (Fig. 1). Lesions, which were visible on both surfaces of the leaf, varied in number from few to many and coalesced when numerous. Severe infections sometimes resulted in defoliation.

Acervuli of *Entomosporium macalatum* Lev., the imperfect stage of *Fabraea maculata* (Lev.) Atk., were produced in the lesions when infected leaves were placed in a moist chamber. Spores collected from infected leaves were used to inoculate plants of *R. indica* and *R. indica* var. *rosea* and the Yeddo-Hawthorn, *R. umbellata* var. *integerrima* Rehd. The plants were then covered with polyethylene bags for 2 days. After 3 weeks, leaf spots appeared on inoculated plants, whereas no spots appeared on check plants.

In nurseries, *R. umbellata* var. *integerrima*, commonly called *R. ovata*, was found to be much more resistant to the leaf spot than plants of *R. indica*. Under conditions favorable to the disease, only a few spots or none at all were found to develop on leaves of the former while leaves of the latter were heavily infected. Inoculation tests also showed *R. umbellata* to be more resistant.

Entomosporium maculatum commonly attacks many plants belonging to the Rosaceae and, according to Weiss[1], has been reported on pear, hawthorn, service berry, quince, medlar, loquat, Christmas berry, cotoneaster, mountain ash, and several crab apples. Although it has been reported on *Rhaphiolepis indica* from Australia[2] and was also reported on *R. Delacouri* Andre from Argentina,[3] this is believed to be the first report of the disease in this country on *R. indica* and the first report of the disease on *R. umbellata* var. *integerrima*.—Department of Plant Pathology, University of California, Berkeley 4, California.

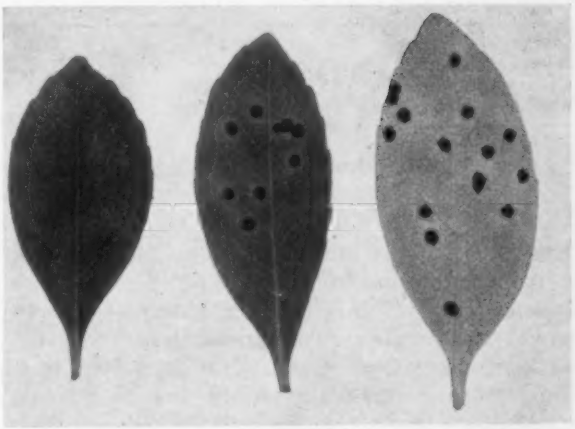

FIG. 1. Leaves of *Rhaphiolepis indica*. Left, healthy; center and right, respectively, upper and lower surfaces of leaves infected with *Entomosporium maculatum*.

Disappearance of Blackrot Symptoms in Cabbage Seedlings.[1] DOUGLAS C. BAIN. In an earlier paper, the writer[2] arbitrarily classed certain varieties of cabbage into blackrot (*Xanthomonas campestris* (E. F. Sm.) Dows.) resistant and susceptible classes on the basis of percentage diseased seedlings from inoculated seed. Wisconsin All Seasons and Copenhagen Market were thus considered to be resistant and susceptible, respectively. During the tests reported, it was observed that seedlings of Wisconsin All Seasons with diseased cotyledons frequently did not develop external symptoms of blackrot later in true leaves even under favorable temperature and moisture conditions. Seedlings of the susceptible variety, however, usually developed severe symptoms in true leaves after cotyledonary infection was apparent. Also, occasional plants of either variety with healthy cotyledons developed blackrot symptoms later in true leaves.

Disappearance of blackrot symptoms in seedlings and early plant stages was pointed out by Walker[3] in 1941. According to this report, external symptoms disappeared (apparently by shedding of diseased parts) and, during cool temperatures at this stage of growth, the pathogen developed slowly and internally without causing external symptoms. Later, however, the temperature increased and became favorable for rapid

[1] Weiss, F. 1940-42. Check list revision of diseases of economic plants. V. 1. Trees, shrubs and woody plants. U. S. Dept. Agr. Pl. Dis. Reptr. 25 and 26.

[2] Anonymous. 1948. New plant diseases. Notes contributed by the biological branch. Agr. Gaz. New South Wales 59: 32-36, 46.

[3] U. S. D. A. 1935. Bureau of Plant Quarantine service and regulatory announcements. Lists of intercepted plant pests. 1934. 84 p.

[1] Journal Article No. 413, Mississippi Agricultural Experiment Station.

[2] Bain, D. C. 1951. Observations on resistance to blackrot in cabbage. U. S. D. A. Pl. Dis. Reptr. 35: 200-205.

[3] Walker, J. C. 1941. Origin of cabbage blackrot epidemics. U. S. D. A. Pl. Dis. Reptr. 25: 91-94.

TABLE 1.—*The development of blackrot symptoms in cabbage plants originating from inoculated seed. Summary of results of 3 tests*

Variety		No. plants killed	No. plants with leaf symptoms	No. plants without external symptoms but blackrot in bundles	Plants healthy	Percentage plants with blackrot
Copenhagen Market	BR[a]	63	204	0	33	89.0
Copenhagen Market	H[b]	0	17	2	75	20.2
Wisconsin All Seasons	BR[a]	12	93	1	155	40.6
Wisconsin All Seasons	H[b]	1	10	2	241	5.1

[a] Cotyledons with blackrot symptoms.
[b] Plants with apparently healthy cotyledons and leaves.

development of the bacteria which resulted ". . . in a 'sudden' epidemic about the time the plants are beginning to head." Appearance of blackrot symptoms several months after seedlings had been inoculated was reported by Cook et al.[4] Of 400 inoculated seedlings, about 300 plants remained free of external symptoms of blackrot on through the heading stage. These were placed in cold storage to induce bolting. Five of these apparently healthy plants developed blackrot symptoms after bolting. Others showed slight vascular discoloration but tissue platings did not reveal presence of the pathogen. Since an understanding of these phenomena was of importance in evaluating resistance, further investigation was considered desirable.

Commercial seed of Wisconsin All Seasons and Copenhagen Market were inoculated, germinated in Petri dishes, and potted in the manner previously described.[5] As soon as blackrot symptoms appeared in cotyledons, a certain number of diseased plants were potted individually. About 3 weeks after emergence, plants that appeared to be free of disease were also potted. As will be seen later, the percentage of plants with diseased cotyledons was not indicative, in either variety, of the percentage that eventually developed typical blackrot symptoms. Because of the higher degree of susceptibility of Copenhagen Market, it was difficult to obtain a satisfactory number of healthy plants of this variety. All plants were kept in the greenhouse at temperatures above 21°C., and were watered frequently. About 60 days after planting, final notes were taken. Three tests were made.

Results of the 3 tests did not vary greatly and substantiated previous observations. Eighty-nine per cent of plants from the susceptible variety with diseased cotyledons developed typical symptoms of blackrot in true leaves and 21 per cent of these were killed (Table 1). The remaining 11 per cent were free of disease symptoms. A little over 20 per cent of plants with healthy cotyledons later showed blackrot symptoms in leaves.

Plants of Wisconsin All Seasons reacted quite differently. Only 41 per cent of plants with diseased cotyledons showed symptoms in true leaves and only 12 per cent of these diseased plants were killed. Five per cent of plants with healthy cotyledons later became diseased.

These results clearly demonstrated that symptoms of blackrot failed to develop in leaves of some plants that had disease symptoms in cotyledons. The percentage of plants exhibiting this phenomenon was much higher in the variety that was considered to be resistant. Killing of diseased plants was also considerably less in Wisconsin All Seasons. Since the environment was conducive to development of the pathogen, apparently disappearance of symptoms was due to differences in varietal (and individuals within varieties) reaction to the blackrot organism rather than to failure of the pathogen to develop because of unfavorable temperatures. That symptoms appeared in leaves of plants not showing cotyledonary infection was also demonstrated. There was a notable difference in varietal reaction also in this respect. Considering these results and those reported by Cook et al, it would appear that plants can be infected in the seedling stage without showing symptoms in a reasonable length of time. It is evident that seed inoculation is an effective means of evaluating resistance but other methods must be employed for accurate determinations. —Truck Crops Branch Experiment Station, Crystal Springs, Mississippi.

Three unusual manifestations of cane gall on cultivated blackberry.[1] EDWARD K. VAUGHAN.[2] Cane gall, caused by *Agrobacterium rubi* (Hildebrand), ordinarily appears on fruiting canes of most varieties of blackberry as rough ridges or elongated protuberances

[4] Cook, A. A., R. H. Larson, and J. C. Walker. 1952. Relation of the blackrot pathogen to cabbage seed. Phytopathology 42: 316-319.
[5] Bain, D. C. 1952. Reaction of brassica seedlings to blackrot. Phytopathology 42: 497-500.

[1] Approved for publication by the Director of the Oregon Agricultural Experiment Station, Contribution of the Department of Botany and Plant Pathology. The assistance of Richard Stace-Smith and Paul H. Wooley, Graduate Students at Oregon State College, is gratefully acknowledged.
[2] Plant Pathologist, Oregon Agricultural Experiment Station.

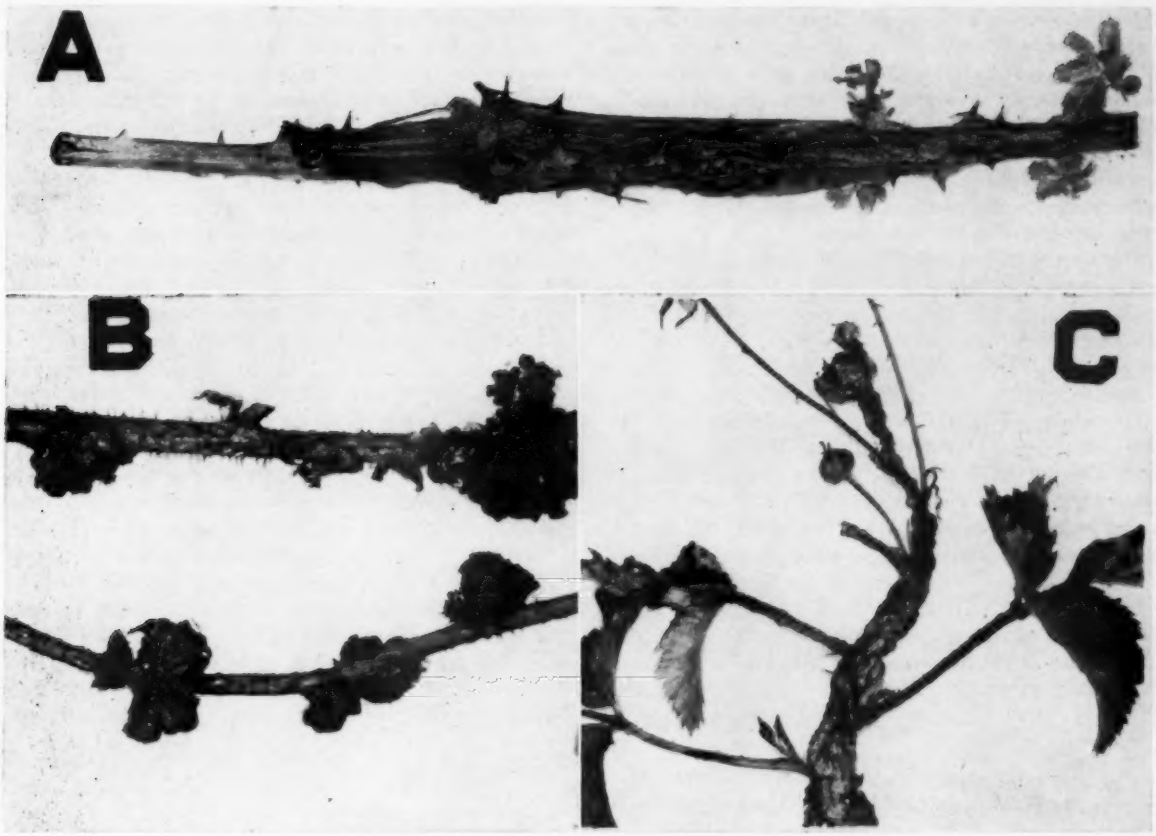

FIG. 1. Cane gall on cultivated blackberries. A) Ordinary elongated gall on Himalaya blackberry, B) roughly spherical galls on boysenberry, C) cane gall affecting petiole, leaf, pedicel, and flower of nectarberry. All are natural infections.—Photographs by H. H. Millsap.

of whitish, granular gall tissue. Enlargement of the galls frequently causes the canes to split open and dry out. Severely injured canes usually produce small, seedy berries. Hildebrand[3] states that the galls are "most abundant on the lower part of the cane but appear also on the small terminal branches." In Oregon, galls on the lower part of the canes are more common than those in the top part of the plant especially following periods of alternate freezing and thawing during late winter.

Three unusual manifestations of cane gall were noted in 1952 and 1953; an irregular type which affected the base of the fruit spurs of Himalaya blackberry, a roughly spherical type which occurs on boysenberry canes, and natural infections of the foliage and flowers of Nectarberry. In comparisons based on physiologic studies, the bacteria involved in these types were indistinguishable from one another, and from those of the ordinary cane gall. The morphological differences of the galls are presumed to be the result of environmental factors, rather than genetic or other variations of the causal organism.

In June, 1952, Himalaya blackberries in the Powell Valley area, southeast of Portland, were severely injured by cane galls which appeared at the base of almost every fruit spur, and were as abundant at the extremity of the canes as near the ground. The galls, which closely resembled those pictured by Bennett[4] in Michigan on black raspberry, were large, sometimes elongated but more often irregular in shape. They caused such severe losses that the berries in many fields were not harvested.

It is common practice in this area to cut the Himalaya variety back to 6 or 7 strong canes, the branches being cut off 4 in. from the main cane, leaving stubs for fruit production. In fields where the plants were severely injured, 100 per cent of the stubs that were left when the canes were pruned were also affected, suggesting that the pruning shears transmitted the infection. Subsequent investigation has shown, however, that this is not true. Sterilization of the pruning shears with corrosive sublimate between cuts did not

[3] Hildebrand, E. M. 1940. Cane gall of brambles caused by *Phytomonas rubi* N. Sp. Jour. Agr. Res. 61:685-696.

[4] Bennett, C. W. 1928. Michigan raspberry diseases. Michigan Agr. Exp. Sta. Special Bul. 178.

affect the amount of cane gall that developed the following season. Furthermore, although bacteria could be isolated readily from the fresh galls in June, numerous attempts to isolate them from the old galls in September and October failed. This indicates that old galls are probably not an important source of inoculum and that cutting of old, infected canes is not likely to contaminate the pruning shears and lead to subsequent infections on the new canes.

The source of inoculum for the aerial galls has not been determined, however. During fall and winter isolations from young tips which had taken root, from new canes that were in contact with the soil, and from the soil directly beneath plants which had been severely infected have all failed to yield the causal bacteria.

The winter of 1952-53 was mild and there were few days when the temperature dropped below freezing. Since alternate freezing and thawing in late winter are usually necessary for infection, a low incidence of galls was expected. Actually there was almost no cane gall in 1953, even in fields where it caused complete losses in 1952. The only exception was in experimental plots where the plants were trellised in October, 1952. Wherever a cane had been twisted or broken during trellising, a typical, elongated gall appeared. Adjacent plots that were trellised in February and March, 1953, the usual time for pruning and trellising the Himalaya variety in that area, were free from infection. This is in agreement with observations of numerous growers, who insist that much more cane gall develops when trellising is done in the fall than when it is done in late winter. No explanation of the difference is apparent.

In 1953, Boysenberry plants in a few fields near Eugene, Oregon, were affected by cane galls that were more numerous in the upper part of the plant than near the ground. Individual galls were roughly spherical rather than elongated (Fig. 1, B), and 1½–3 in. in diameter. The general appearance was much more like crown gall caused by *Agrobacterium tumefaciens* than ordinary cane gall (Figure 1, A). Yield and quality of berries were reduced appreciably. This type of cane gall has been seen only rarely in western Oregon during the last several years.

In 1952 and 1953, cane gall on Nectarberries on a farm near Corvallis, Oregon, affected not only the canes but all parts of the fruit spurs as well (Fig. 1, C). Bacteria were isolated from galls on petioles, leaves, pedicels, and flowers. This is the only instance in which the writer has seen natural infection by cane gall on leaves and floral parts of *Rubus* in Oregon. Much of the planting has now been abandoned because of the disease.—Department of Botany and Plant Pathology, Oregon State College, Corvallis, Oregon.

NOTICE

A preliminary meeting was held recently to explore the possibility or advisability of developing a uniform system for use in coding biological taxonomic entities. Participants at this exploratory meeting were from the Library of Congress, the United States Department of Agriculture, the Smithsonion Institution, and the Chemical-Biological Coordination Center of the National Research Council.

It is known that several laboratories are entering the results of their experiments on machine or hand-sort punch cards and that at least a few are listing the species of plants or animals tested by means of a code on these punch cards. In order to facilitate the exchange of information between various laboratories, it would seem to be highly desirable for these research groups to use a uniform taxonomy coding system. If the need or usefulness of uniformity is confirmed by sufficient biologists, a start on the formation of a standardized code should be made as soon as possible so that the conversion from individual codes can be accomplished with a minimum of effort.

The interested persons who have been informally discussing the project do not want to set plans in motion for the actual codification unless there is a real or potential need for it. Our immediate problem, therefore, is to determine what areas of biology could be usefully served by a standard taxonomic code. We are, therefore, requesting that those who have worked out or adopted a system for coding taxonomic entities, or who may be conducting work which might benefit from such codification, submit their opinions concerning a standardized biological taxonomy code.

Letters should be addressed to: Dr. G. Congdon Wood, Chemical-Biological Coordination Center, National Research Council, 2101 Constitution Avenue, N.W., Washington 25, D.C.

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In recognition of the many services which the late L. R. Tehon rendered his community particularly through his long and active interest in the Boy Scouts of America, the Arrowhead Council of Boy Scouts seeks to establish a fitting memorial. No decision as to the form of the memorial has been made other than it should appropriately represent his interests and activities. Contributions will be accepted from any individual or organization that wishes to participate in the memorial and may be addressed to Tehon Memorial Committee, Arrowhead Council, No. 117, 614 East Green Street, Champaign, Illinois.

Historical items, of significance to the profession of plant pathology, are being collected and conserved by members of a new permanent committee of the Society. Arrangements are underway to establish a file for material, as it becomes available, with the National Fungus Collections at Beltsville. Records of any kind bearing on this subject, including letters, programs and related documents, will be welcomed and carefully preserved. Material should be sent to committee members, E. L. LeClerg, J. Walter Hendrix, Karl Maramorosch, or John A. Stevenson, Chairman.

Retired pathologist, Harry B. Shaw was honored by the Photographic Society of America and named an associate in recognition of his contributions to photography. Mr. Shaw was recently re-elected secretary-treasurer of the American Society of Photographic Art, holding that post since 1949. He has also been president of the National Photographic Society of Washington since 1950. Before his retirement, Mr. Shaw was Pathologist and Plant Quarantine Inspector in Charge of Information Service, Foreign Plant Quarantines.

Phytopathologist, Russell B. Stevens has been appointed executive secretary of the Biology Council of the Division of Biology and Agriculture, National Academy of Sciences, National Research Council.

Virology, a new journal devoted exclusively to publishing articles on the biological, biochemical and biophysical aspects of virus science is announced by Academic Press, Inc. It is planned to publish one volume per year consisting of five issues, totaling approximately 400 pages. The first volume is tentatively scheduled for release in April. It will be priced at 9 dollars.

For sale, complete set of Phytopathology (Volumes 1-42) bound in brown buckram with Volume 43 and current numbers unbound. The bound volumes are priced at 10 dollars per volume and the unbound volumes at 8 dollars. If interested, contact Dr. Leland Shanor, Department of Botany, University of Illinois, Urbana, Illinois.

The Potomac Division will hold its annual meeting March 3 and 4 at the Plant Industry Station, Beltsville, Maryland. In addition to presentation of research papers, a symposium, "Fungicides—Past, Present, and Future", will be held. The symposium will be moderated by Dr. J. W. Heuberger and the speakers will be: Dr. George L. McNew, Director of the Boyce Thompson Institute for Plant Research, who will speak on the "History and Philosophy of the Use of Fungicides"; Dr. Hubert Martin, formerly of England, now Director of the Science Service Laboratory, London, Ontario, will speak on the topic, "The mode of action of Fungicides"; Dr. T. Walter Reed, Assistant Manager of Research, California Spray Chemical Corporation, with the topic, "Problems Encountered in the Commercial Production and Formulation of Fungicides"; and Mr. Lea S. Hitchner, Executive Secretary of the National Agricultural Chemicals Association, will discuss "Legal and Moral Aspects Encountered in the Use of Fungicides." Mr. Hitchner will place emphasis on the recently passed Miller Bill. The speaker for the annual banquet the evening of March 3 will be Dr. Karl D. Butler, Executive Secretary of the National Institute of Animal Agriculture and Farm Counselor to the Avco Manufacturing Corporation. Dr. Butler's topic will be "The Future of Agriculture".

Editor: K. W. KREITLOW, Plant Industry Station, Beltsville, Md.

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